The Effect of Hypoxia and Hypoxia-Associated Pathways in the Regulation of Antitumor Response: Friends or Foes?

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Hypoxia is an environmental stressor that is instigated by low oxygen availability. It fuels the progression of solid tumors by driving tumor plasticity, heterogeneity, stemness and genomic instability. Hypoxia metabolically reprograms the tumor microenvironment (TME), adding insult to injury to the acidic, nutrient deprived and poorly vascularized conditions that act to dampen immune cell function. Through its impact on key cancer hallmarks and by creating a physical barrier conducive to tumor survival, hypoxia modulates tumor cell escape from the mounted immune response. The tumor cell-immune cell crosstalk in the context of a hypoxic TME tips the balance towards a cold and immunosuppressed microenvironment that is resistant to immune checkpoint inhibitors (ICI). Nonetheless, evidence is emerging that could make hypoxia an asset for improving response to ICI. Tackling the tumor immune contexture has taken on an *in silico*, digitalized approach with an increasing number of studies applying bioinformatics to deconvolute the cellular and non-cellular elements of the TME. Such approaches have additionally been combined with signature-based proxies of hypoxia to further dissect the turbulent hypoxia-immune relationship. In this review we will be highlighting the mechanisms by which hypoxia impacts immune cell functions and how that could translate to predicting response to immunotherapy in an era of machine learning and computational biology.

Keywords: hypoxia, tumor microenvironment, immune microenvironment, tumor plasticity, genetic instability, immunogenicity, hypoxia signature

INTRODUCTION

Solid tumors manifest in a microenvironment that harbors an array of cellular and non-cellular factors, cycling through various environmental pressures, which contribute to shaping a tumor's immunological features (1). Hypoxia is an early event in tumor evolution that has been shown to both directly and indirectly impact this tumor immune microenvironment (TIME), with much of

the evidence leaning towards an immunosuppressive influence (2–4). In addition, this condition of low oxygen is implicated in enabling tumor aggressiveness by providing tumor cells with a metabolic advantage (5) and by modulating autophagy (6). Hypoxia also promotes stemness and epithelial-mesenchymal transition (EMT) (7), genomic instability (8), and angiogenesis (9), thus contributing to all cancer hallmarks.

The repercussions of hypoxia in the TME occur through both hypoxia inducible factor (HIF)-dependent and independent mechanisms. HIF proteins are heterodimers composed of a constitutive β-subunit and an inducible α-subunit (HIF-1α, HIF-2α or HIF-3α). Under normal oxygen tension, HIF-1α and HIF-2α subunits are hydroxylated by prolyl hydroxylase (PHD), resulting in their subsequent ubiquitylation by the Von Hippel-Lindau tumor-suppressor protein (VHL), a component of an E3 ubiquitin ligase complex, and degradation by the proteasome (10). In hypoxic cells, HIF proteins are stabilized and in turn regulate the transcription of downstream genes, thereby modulating the microenvironmental stimuli within a tumor. The result is an acidic, nutrient deprived and immune-hostile microenvironment that is resistant to immunotherapy (2, 11). Indeed, limiting hypoxia in the TME in preclinical models has shown considerable improvement in the response to immune checkpoint inhibitors (ICI) (12, 13).

While there is emerging evidence of HIFs enhancing the activation status of immune cells in the TME (14–16), hypoxia is also known to mitigate their infiltration rate and function (3, 4). The TME comprises a slew of immune cell types, including those derived from the innate arm of the immune system, namely natural killer cells (NK) cells, macrophages and dendritic cells (DCs); as well as those belonging to the adaptive arm, including CD8+ effector T cells and CD4+ helper T cells. NK cells provide major histocompatibility complex (MHC)-unrestricted cytotoxicity against tumor cells (17). They also contribute to the sensitization of tumor cells to effector T cell killing by secreting the interferon, IFN-γ which acts on tumor cells upregulating their MHC and immunoproteasome expression (18, 19). With respect to macrophages, there is mounting evidence on the marked heterogeneity among populations resulting in a spectrum of macrophage subtypes with varying transcriptional sates (20, 21). This is contrary to the original binary model, wherein it is generally accepted that tumor associated macrophages (TAMs) display the so-called M2-like phenotype, which exhibits pro-tumorigenic features; while M1 macrophages have tumoricidal function and are classically activated (20). Dendritic cells are another subset of innate immune cells, classified as antigen presenting cells, that given the presence of co-stimulatory molecules and the correct cytokine environment, work to prime and activate T cells against tumor cells (22, 23). With respect to adaptive immune cells, the CD8+ effector T cells, or cytolytic T cells (CTLs) take the reigns as they are involved in direct tumor cell death through the induction of apoptosis and through cytokine secretion (24). The CD4+ T cells, on the other hand, exist as various subsets, among which the T helper 1 (Th1) subset is the most studied and is known to contribute antitumor activity by both direct killing

and cytokine release; while regulatory T cells (Treg) and T helper 2 (Th2) cells constitute immunosuppressive subsets (24).

Hypoxia's involvement in reprogramming the TME to one that is conducive to immune resistance has been evidenced multiple times, however exploring the intricacies of the hypoxia-immune cell relationship *in vivo* has been a challenge. The coupling of signatures reflecting the degree of a tumor's hypoxic state with computational algorithms that can delineate its respective immune composition could uncover unexplored pathways and mechanisms of immune resistance. This is crucial given that an in depth understanding of the interplay between hypoxia-driven tumor cell remodeling and the immune contexture could aid in the betterment of patient response to immunotherapy. In this review, a link will be woven between the survival strategies taken up by tumor cells under hypoxic conditions and their impact on the immune microenvironment. In addition, recent findings from *in silico* analysis and the application of such tools to address hypoxia and the TIME will be discussed.

HYPOXIA METABOLICALLY REPROGRAMS THE TUMOR MICROENVIRONMENT EXCLUDING AND PERTURBING IMMUNE CELL FUNCTION

The increased requirement for oxygen and nutrients within the hypoxic TME breeds a metabolic switch that works to nurture tumor survival, while posing as a functional barrier to the sustainability and activity of an anti-tumor immune response. When levels of molecular oxygen become too low to sustain mitochondrial adenosine triphosphate (ATP) production, a transition occurs from oxidative phosphorylation to glycolysis. This is supported by HIF-1α-induced upregulation of glucose transporters, which enhance the influx of glucose, that is in turn shuttled through the glycolytic pathway thanks to the HIF-1-mediated transactivation of key regulatory glycolytic enzymes, while inhibiting the tricarboxylic acid (TCA) cycle (5). As a byproduct of glycolysis, hypoxic cells concomitantly experience high levels of intracellular lactate and hydrogen ions. To overcome the eventual acidification of the cell, HIF-1α induces the expression of transporters and carbonic anhydrases to expel them (5, 25). The net effect is a glucose depleted and acidic TME with a pH as low as 5.8 to 6.5 (5, 26) and lactate concentrations reaching up to 30 mM, that is ten times higher than normal tissue (27). In such a TME, the anti-tumorigenic function of immune-activating cells is thwarted, while that of immunosuppressive cells is advocated.

Just like cancer cells, cytotoxic T cells also rely on glucose for aerobic glycolysis, which is itself necessary for their effector function (28); however, in the TME tumor cells outcompete T cells for glucose, thus inhibiting their antitumor activity (29). Glucose metabolism is further integral to the inflammatory phenotype in macrophages, the maturation and function of dendritic cells and NK cell activation (24, 30). Paradoxically, the immunosuppressive Tregs gain a metabolic advantage in a

glucose deprived TME since they are less dependent on glucose as an energy source (31). Similarly, while Treg is resistant to the high lactate levels in the extracellular milieu (31), both NK cells and CD8+ T cells are encumbered by it. *In vitro* studies have shown that NK cell cytotoxicity and cytokine production are suppressed by high lactate and low pH levels (32, 33), as is CTL survival and function (4, 33). Lactate was shown to perturb DC maturation (34) and to drive Treg polarization from naïve T cells (35). Other immunosuppressive cells are likewise affected by the high lactate concentrations. The infiltration level of the T cell- and NK cell- suppressor, myeloid-derived suppressor cells (MDSC) is increased by tumor lactate (32). Tumor-derived lactate also induces the polarization of TAMs into the M2-like immunosuppressive phenotype (36) and treatment of a macrophage cell line with lactate promoted gain of M2-like features and downregulated the expression of cytokines, TNF-α and IL-12, secreted by M1 TAM (37). In addition, the acid-labile interferon, IFN-γ is rendered dysfunctional in this hostile TME, which in turn halts the maturation of anti-tumor M1 macrophages and promotes the differentiation of T helper cells to tumor promoting Th2 cells (38).

Apart from its impact on glucose metabolism, hypoxia interferes with amino acid and lipid metabolism, which are also essential for fueling cancer cell's survival and modulating the immune contexture. The availability of the nonessential amino acid, glutamine, as well as other amino acids including tryptophan and arginine, which are vital for T cell function, can additionally be modulated by hypoxia. In particular, *in vitro* experiments showed that the deprivation of glucose or glutamine, which results in low α-ketoglutarate (α-KG), skewed CD4+ T cell differentiation in favor of immunosuppressive Treg cells (39). On the other hand, glutamine blockade *in vitro* and in tumor-bearing mice left CD8+ T cells metabolically intact and functional, while suppressing oxidative and glycolytic metabolism of cancer cells, leading to nutrient depletion, attenuated hypoxia, and acidosis in mice (40). This divergent response was attributed to the effector T cells using alternative sources as supplement for their long-lived, highly activated phenotype (24, 40). The opposite changes in cancer cells and effector T cells touch on a metabolic plasticity among the two that can be harnessed as an additional checkpoint for immunotherapy (24, 40). With respect to tryptophan, hypoxia has been shown to induce the expression of the rate limiting enzyme in its catabolism, indoleamine 2,3-dioxygenase 1 (IDO1). In macrophages, this resulted in suppressed T cell proliferation, coupled with enhanced expansion of immunosuppressive Tregs (41). Furthermore, IDO1 depletes tryptophan inducing an amino acid starvation response that promotes T cell anergy (42). While hypoxia was also shown to induce IDO-1 in DC (43), an opposite effect was reported in cancer cell lines of ovarian (44), cervical and glioblastoma (45) origins. Furthermore, the functional ortholog of IDO, TDO2 (tryptophan-2,3-dioxygenase), was found to be significantly downregulated in a HIF-1α dependent manner in glioblastoma cells exposed to hypoxia (46). TDO2 expressing cells in hypoxia were able to rescue T cell proliferation that is otherwise

suppressed under normoxic conditions (46). The interplay between hypoxia and tryptophan metabolism is clearly riddled with controversial evidence, nonetheless, targeting IDO, as well as other players in the tryptophan catabolic pathway is being investigated in various clinical trials, alone or in combination with immune checkpoint inhibitors (47). In terms of arginine, it is metabolized rapidly by activated T cells and supplementing them with increased arginine levels was shown to enhance their anti-tumor activity *in vivo* (48). On the other hand, low arginine levels have been shown to suppress activating receptors of NK cells, like NKp30 and NKp46, to reduce the ability of NKs to produce IFN-γ and to impair their proliferation (24). Hypoxia has been shown to upregulate the expression of the two main enzymes in arginine metabolism, arginase 1 (ARG1) and the inducible nitric oxide synthase (iNOS), on MDSCs. This was in a HIF-1α-dependent manner and resulted in the differentiation of MDSCs into M2-like TAMs (49). MDSCs thereby compete with T cells for the utilization of this crucial amino acid, inhibiting T cell proliferation (50). Furthermore, *in vitro* coculture of macrophages with T cells in hypoxia promoted an increase in iNOS and ARG1 that resulted in T cell inhibition (51). Interestingly, another mechanism that leads to the induction of ARG1 on the surface of MDSCs may also be modulated by HIF1 and that is through the increased production of prostaglandin E (PGE). The inducible cyclooxygenase-2 (COX-2) in tumor cells leads to increased expression of PGE2, which has been shown to maintain the expression of ARG1 on the surface of MDSCs (52). COX-2 and the increase in PGE2 has also been shown to occur in a HIF-1α dependent manner, inhibiting the maturation of DC and enhancing the suppressive capacity of Tregs (53). Therefore, hypoxia could be compounding the depletion of arginine in the TME and the accompanying immunosuppression.

Along with its interference with nutrient uptake and their metabolism, hypoxia has been widely implicated in sending ATP metabolism into overdrive, which further feeds into an immunosuppressive outcome. In an inflammatory setting, extracellular ATP can be released by stressed and dying cells as well as activated monocytes and is involved in immune activation (54). The safety switch to halt the activated immune response and prevent damage of healthy tissue involves the phosphohydrolysis of extracellular ATP to adenosine; a process predominantly regulated by the two membrane-bound nucleotidases, CD39 and CD73 (54). Of interest, both ectonucleotidases are abundantly expressed in the TME and are additionally upregulated through a HIF-1α dependent mechanism in hypoxia (55). This is highly relevant in amplifying the immunosuppressive nature of the hypoxic TME, since adenosine possesses immune-dampening properties, repressing T cell effector function while stabilizing the suppressive function of Tregs (56). The immunosuppressive effects of extracellular adenosine have been well documented and are executed through the binding of this ligand to the Gs-protein-coupled receptors A2aR, expressed on the surface of monocytes, lymphocytes, NK cells and DC, as well as A2bR, which is most prominently expressed on DCs and macrophages (54, 56). Through these two purinergic receptors, adenosine

triggers cyclic AMP (cAMP) accumulation. Within immune effector cells an increase in this intracellular signaling molecule results in an accumulation of an array of immunosuppressive molecules, including IL-10, TGF-β, PD-1 and CTLA-4, as well as the downregulation of key effector factors, such as IL-2, IFN-γ and perforin, which ordinarily participate in a pro-immune response (57). Hypoxia can further modulate adenosine levels through HIF-1α-dependent inhibition of adenosine kinase activity required to generate adenosine monophosphate (AMP), which in turn maximizes extracellular adenosine accumulation and depletes ATP levels in the cell (58). Indeed, the Hypoxia-Adenosine-Adenosine receptor axis represents various pharmacological targets and preclinical data support the rationale of combining A2aR blockade with hypoxia targeting strategies to reinvigorate the NK and T cell mediated anti-tumor immune response (59). Present data thereby suggests supplementing this combinational approach to current immunotherapeutic options to potentiate their efficacy (59).

HYPOXIA-MEDIATED AUTOPHAGY PLAYS A DOUBLE ROLE IN THE IMMUNE RESPONSE

There is broad consensus that hypoxic stress in the tumor microenvironment activates autophagy mediated adaptation to low oxygen, however, autophagy outcome is still controversial and is observed as a double agent both promoting or suppressing tumor development commensurate to tumor type and staging which is strongly correlated to the therapeutic response. In fact, autophagy can mediate adaptive survival response to hypoxia (60–62) or a nonapoptotic programmed cell death called autophagy cell death (63, 64). Clinical data suggests a direct correlation between autophagy influx and tumor development. High Beclin-1 expression was linked with poor prognosis in advanced human nasopharyngeal carcinoma and colorectal adenocarcinoma samples (65, 66). Moreover, high autophagy turnover tumors were less sensitive to treatments in comparison with low autophagy turnover (67). As hypoxia-mediated autophagy induces more resistance to tumors in response to therapies than normoxic cells (68), a dual synergic treatment with autophagy inhibitors is suggested (69). During hypoxia, autophagy is induced through the activation of the HIF-1α which upregulated BCL2 Interacting Protein 3 (BNIP3) and BNIP3L, rendering Beclin-1 free to promote its interaction with VPS34 and the formation of autophagolysosomes (6). Alternatively, hypoxia induced autophagy can be mediated independently of HIF-1α through the Unfolded Protein Response (UPR) (70) or through the energy sensor AMP-activated protein kinase (AMPK) (64).

It has now been well documented that tumor lesions form, progress, and respond to therapy in the setting of a complicated interaction with the host immune system (71, 72). Data from genetically engineered mouse models demonstrated that autophagy influx influences the tumor cells as well as the immune cells in the TME (73, 74). Thus, autophagy machinery is

suggested as a potential beneficial pharmacological and genetic target to mitigate anti-tumor immune responses (75–79) with some successful preclinical data. Notably, immune cells' activation, differentiation and proliferation can be modulated by autophagy, which mediates promotion or inhibition of tumor development. Under hypoxic condition, as in tumors, immune cells experience hypoxia and have to adjust their metabolic needs and may do so through autophagy machinery which plays a plethora of action of immune cells to regulate anti-tumor immune reaction. CD8+ cells differentiation to cytotoxic T lymphocytes (80), their infiltrating and stemness preservation (81), T cells differentiation to Th cells, iNKT cells survival, differentiation and proliferation (82), DCs and B cells development (82), Treg cells survival, stability and immune tolerance (83), monocytes differentiation into macrophages and polarization and the number of macrophages as well (84), MDSCs growth and the establishment of T cell memory (85–87) are enhanced by autophagy. However, autophagy negatively regulates neutrophils development and induces their degradation (88).

Similarly to its dual impact on tumors development, autophagy can be observed as immune-simulator or immune-suppressor in the context of immune-mediated tumor elimination (88). It even becomes more complex when tumors are submitted to hypoxic stress. Understanding the contribution of hypoxia-induced autophagy in immune response to tumors is instrumental for better shaping therapeutic strategies. Thus, many studies showed the implication of multiple signaling pathways in hypoxia inducing autophagy to downregulate immune responses. Hypoxia-induced autophagy can attenuate NK cells anti-tumor activity. Loss of HIF-1α in NK cells blocks tumor growth (89) and hypoxia upregulation of HIF-1α in NK cells is dependent on PI3K/mTOR signaling pathway activation in response to cytokine receptor gamma chain, reducing NK cells tumor suppressive function (90). Moreover, hypoxia can modify the transcriptome of NK cells, regulating their immunoactivity and influencing their migration which may profoundly influence their infiltrating capacity in tumor tissues (91). Once X-irradiated, NK cells became more resistant and maintain killing capacity under hypoxic conditions (92). Recently, data demonstrated a strong correlation between attenuated NK cell cytotoxicity and a decreasing level of phosphorylated STAT3 and ERK through protein tyrosine phosphatase SHP-1 (Src homology region 2 domain-containing phosphatase-1) activation (93). Both STAT3 and ERK phosphorylation is increased in pre-activated hypoxic NK cells which restores their proliferation under hypoxic conditions (94). In contrast, hypoxia-induced autophagy attenuates CTL-mediated tumor degradation by activating the Src Kinase, which phosphorylates STAT3 in a HIF-1α dependent manner (95, 96). Simultaneously, HIF-1α induces autophagy thought the Beclin-1- BNIP3- Bcl-2 axis, resulting in the degradation of the SQSTM1/p62 protein responsible for the degradation of p-STAT3 leading to its accumulation in cells (97, 98) and preventing CTL attacks. Concomitantly, STAT3 expression promotes HIF-1α expression and modulates hypoxia-induced EMT in esophageal squamous cell cancer (99). Moreover, hypoxia-induced

autophagy degrades NK-derived Granzyme B in tumor cells through the autophagy sensor Inositol 1,4,5-Trisphosphate Receptor Type 1 (ITPR1), thus impairing NK-mediated tumor cell degradation (100). Furthermore, hypoxia-induced autophagy has been associated with destabilization of the immune synapse between the NK and the tumor cells through decreasing the level of connexin 43 leading to impairment of NK killing efficacy (101, 102).

HYPOXIA-DRIVEN TUMOR PLASTICITY AND HETEROGENEITY INCITE IMMUNE ESCAPE

Epithelial to Mesenchymal Transition (EMT) and Stemness in the TME

Optimum oxygen levels are essential to maintain tissue homeostasis. When oxygen sensing mechanisms or when oxygen levels decrease, a cascade of molecular events escalates a multitude of responses. Stabilization of HIF-1α ensues molecular changes that initiate EMT. EMT is characterized by an increase in cell migration, invasion, production of extracellular matrix (ECM) and resistance to apoptosis (103). Specific transcription factors are activated by HIF-1α to mediate EMT phenotypes, these include SNAIL SLUG, TWIST1, ZEB1, SIP1/ZEB2 (104). Additional pathways that have been shown to be involved in the hypoxia-mediated EMT include TGF-β, Wnt/β-catenin, hedgehog and Notch (104).

The process of EMT in cancer cells endows them with stem cell features. These newly formed cancer stem cells (CSCs) result in a heterogeneous cancer cell population. CSCs just like normal tissue stem cells can adapt a quiescent cellular state, characterized by a cell cycle arrest with reduced metabolic activities (105). In addition, CSCs have the capacity to self-renew and differentiate, as such they are credited for tumor growth, invasive growth, and metastasis at distal sites (106). The quiescent state of CSCs contributes to their resistance to therapeutic drugs (105). However, targeting CSCs requires an understanding of the several developmental signaling pathways that function to mediate and maintain their self-renewal and differentiation, these include TGF-β/BMP, Notch, Wnt, Hedgehog, FGF, and IGF (107). These signaling pathways are interconnected and overlapping and are present at crossroads that feedback into the hypoxia axis: TGF-β/SMAD3 pathway can be activated by HIF1 and at the same time it results in the stabilization of HIF1 by suppressing PHD levels (104). Notch interacts with HIF-1α to turn on expression of genes important in maintaining the undifferentiated cell state (108). In addition, Notch signaling regulates SNAI1 as well as hypoxia-induced cell motility and invasion (108). Wnt/β-catenin signaling has a pro-EMT effect in cells under hypoxia (109). Furthermore, HIF-1α knockdown abolishes hedgehog pathway activation (110). Finally, FGF induces HIF-1α expression (111).

Identifying cancer stem cells relies on the expression of unique markers on their cell surface. Depending on the cancer type these include EpCAM, ALDHA1, Lgr5, CD13, CD24, CD26, CD47, CD49f/Integrin alpha 6, CD66c, CD90, CD166, CD271, CD105, CD44, CD133, CD117/c-kit, CD138, CD151 and CD166 (112). CD44 and CD133 are the most widely used markers. CD44 is a transmembrane glycoprotein that is expressed in solid and hematological cancers, it mediates stromal interaction and has different activation states upon binding to its ligand hyaluronan (HA). In this context, CD44 has been shown to be active on cancer cells and not in normal cells (113). CD44 constitutes a potential marker to enhance targeted therapy, indeed in breast cancer CD44-doxorubicin conjugated aptamers inhibited selective cell proliferation of CD44 expressing cells (114). CD133 is a transmembrane glycoprotein expressed in several tumors. A variety of promising immunotherapeutic strategies have been developed to target CD133 expressing cells (115).

Impact of Plasticity and Heterogeneity on Tumor Immune Escape

Hypoxia-driven tumor plasticity and heterogeneity may have substantial impact on immunosuppression and cancer immune evasion (116). The pioneer study of Ye and colleagues previously revealed that hypoxia-induced EMT of hepatocellular carcinoma cells can promote an immunosuppressive TME by stimulating the release of the CCL20, leading to the production of IDO by monocyte-derived macrophages, which in turn suppressed T cell proliferation and promoted the expansion of immunosuppressive regulatory T cells (41). In fact, there are many known potential factors such as TGF-β contributing to hypoxia-driven tumor immune escape (3, 7) evoking features of cancer stem cells and tumor epithelial-mesenchymal plasticity. We previously showed that the stemness-associated transcription factor NANOG is induced by hypoxic stress; not only conferring stemness properties to carcinoma cells, but it also increases TGF-β expression and secretion, thereby promoting infiltration of immunosuppressive cells in the murine B16 melanoma model (117). We also showed that hypoxic stress can promote EMT programs enhancing immune evasion of NSCLC carcinoma cells (118). In the human IGR-Heu model, the tumor population was found to be highly heterogeneous following hypoxic stress, with an important fraction of cells conserving marked epithelial features. The mesenchymal cancer clones were found to have increased intrinsic TGF-β pathway activity and increased capacity to resist attacks by immune cytotoxic effector cells compared to the more epithelial clones, as reflected by reduced cancer cell susceptibility to CTL and NK cell-mediated lysis. To note, heterogeneity also exists within the mesenchymal clones. For instance, the expression of the receptor tyrosine kinase AXL could mark cancer clones with pronounced immune evasion capacity in association with reduced expression of ICAM1, ULBP1, and MHC class I levels in cells (119). It is interesting to consider that AXL expression can be upregulated by many intrinsic as well as extrinsic factors including hypoxia (120, 121). Published data have been unclear across different cancer systems and models. Research is still needed to decrypt the regulatory events controlling the expression of AXL and more generally of the TAM (Tyro3 Axl Mertk) family receptors. On the other hand, AXL activity has been shown to

support a hypoxic state in carcinoma cells by stabilizing tumoral HIF-1α through cooperation with HER2 (122). In a murine Her2+ breast cancer model, this cooperative event greatly contributed to shaping an immunosuppressed TME, while Axl targeting led to improving anti-Pd-1 treatment efficacy.

Work by Zhang and colleagues revealed that HIF-1α can stimulate CD47 expression in breast cancer cells gaining stemness features, which also serves as a mechanism to evade phagocytosis by innate immune cells such as macrophages (123). The CD47 SIRP interaction hampers the "eat me signal" on macrophages impairing phagocytosis. Another study found that the CD47 gene is a direct target of EMT-associated transcription factors SNAI1 and ZEB1 (124). Moreover, CD47 and PD-L1 can act synergistically to sustain resistance and immunosuppression (123). Interestingly, carcinoma cells with stemness features certainly exhibit immunogenicity profiles that differ from well-differentiated carcinoma cells with consequences on tumor immunogenicity, neo-antigen expression and the anti-tumor immune response (125). Complex interactions between the different contingents should also be highlighted. For instance, Faget et al. showed that neutrophils in lung tumors alter angiogenesis and immunotherapy efficacy by promoting tumor hypoxia and partial EMT of carcinomas as events of a vicious cycle maintaining an immunosuppressed pro-tumoral microenvironment (126).

Thus, several studies have demonstrated the role of hypoxia mediated-EMT and plasticity on tumor immune escape, although with variability in terms of the mechanisms and cell types involved. It will be important to better integrate the intratumor heterogeneity parameter in future investigations. Another important challenge will be to translate this information into the clinic with safe effective strategies.

Hypoxia-Dependent Modulation of Cancer Cell Glycosylation as a Mediator of Immune Escape

Another aspect of cancer cells that is modified by hypoxia and plays a role in the modulation of the immune response is glycosylation. Addition of glycans is a posttranslational modification that regulates the activity of as many as 50% of human genes, making it one of the most important regulators of gene expression. In tumors, abnormal glycosylation gives rise to a glycol-profile that perpetuates key cancer hallmarks of proliferation, EMT, angiogenesis, invasion, and metastasis (127, 128). Moreover, immune response itself is highly controlled by glycosylation [as reviewed in (129)]. Altered glycan residues on cancer cell surface, usually related to increased or unusual sugar components, give rise to tumor-associated carbohydrate antigens (TACAs) which are weakly immunogenic, and thus may serve as an immune escape strategy (130–132). Glycosyltransferases and glycosidases that add and remove sugar residues, respectively, are highly modulated by hypoxia in a tumor-dependent fashion (127, 128). In addition, hypoxia contributes to increasing specific structures involved in tumor invasion and immune escape (127, 128). In particular, the highly hypoxic muscle-invasive bladder cancer (MIBC) overexpresses the cancer-associated carbohydrate antigen sialyl-

Tn (STn), which has been reported to be at least in part due to a HIF-1α-dependent cell survival strategy that favors cell migration and invasion (133). Recently, through the application of glycoproteomics in bladder cancer, the same group demonstrated cell surface expression of an ordinarily intracellular protein, homer homolog 3 (HOMER3), carrying short-chain O-glycans that are characteristic of membrane proteins (134). They further reported that under glucose deprivation and hypoxic conditions, HOMER3 contributed to the tumor cell's invasive capacity. Of interest, cell-surface expression of this protein was associated with significantly worse survival in MIBC patients. Furthermore, while HOMER3 expression was not cancer-specific, STn and HOMER3 did not co-express in healthy tissue, suggesting that HOMER3-STn could be a tumor-specific biomarker that can be used to target the more aggressive cancer cell populations residing in the hypoxic TME (134). Similarly, some cancer-specific glycoconjugates, like N-glycolyl (NeuGc) GM3 gangliosides, are promising therapeutic targets, as their increased expression is characteristic in tumors and almost not present in healthy human tissues (135). Gangliosides are glycosphingolipids containing sialic acid residues and their expression can be induced by hypoxia (136). While the exact mechanism is unknown, hypoxia has been shown to induce the sialic acid transporter, sialin (137). In addition, despite humans lacking the functional enzyme responsible for N-glycolyl (NeuGc) GM3 synthesis, hypoxia upregulates the succinate dehydrogenase subunit B (SDHB) of the mitochondrial respiratory complex II, which is hypothesized to provide the deleted iron/sulfur catalytic domain of the enzyme, restoring its functionality (138). Gangliosides, and in particular GM3(NeuGc), have been showed to play a key role in suppressing the antitumor immune response and therefore serve as neoantigens that can be targeted by immunotherapy (135). Indeed, given that they can also induce antibody responses, several clinical trials are ongoing to use them as anti-cancer vaccine antigens, as has recently been reviewed (135, 139).

ROLE OF HYPOXIA IN ANGIOGENIC SWITCH AND PRO-ANGIOGENIC MILIEU MODULATION OF IMMUNE RESPONSE

Initially, a growing tumor remains avascularised and relies on the diffusion of oxygen and nutrients from surrounding tissues (140) or reprograms metabolically to survive hostile, O$_2$ and nutrient limited environment (as discussed above). However, upon progression a phenotypic change in cancer cells occurs, termed angiogenic switch when balance of secreted factors moves from anti- towards proangiogenic. This event causes dramatic change in the tumor milieu that primarily induces angiogenesis (141). Previously mentioned HIF-1α stabilization in response to hypoxia activates in cancer cells not only adaptation to low pO$_2$ but also production of one of the most potent proangiogenic factors, vascular endothelial growth factor (VEGF). Other proangiogenic factors secreted by cancer cells include fibroblast growth factor (FGF) family, interleukin-8 (IL-8), epidermal growth factor (EGF) and platelet-derived growth factor

(PDGF) (142). In response to such milieu [and, also hypoxia itself, reviewed elsewhere (143)], in order to supply the growing tumor, endothelial cells (ECs) from surrounding tissues are activated to form new vessels. However, due to dysregulation of proangiogenic response in cancer cells, the forming vessels are disturbed, as a consequence of pathological angiogenesis, contributing even more to cancer progression (144).

Pathological Endothelium and Consequences for Immune Homing

Cancer-associated, pathological vessels are characterized by leakiness and disturbed shape causing uneven vascularization of the tumor mass. Cellular and ECM composition of cancer-associated vessels are altered, which limits their barrier functions causing uncontrolled transport of nutrients, oxygen, and drugs. Consequently, due to abnormalities, newly formed vessels are not able to restore physiological level of oxygen within the tumor, and hypoxia sets in. Impaired perfusion and increased interstitial pressure of cancer vessels were shown to negatively affect leukocyte trafficking (145). Additionally, composition of immune homing receptors is altered in cancer-associated endothelial cells, which affects the infiltration of the tumor with leukocytes. In a steady state, ECs remain quiescent, regulate blood flow and barrier functions of the endothelium, however upon activation, for example in response to inflammation, expression of adhesion molecules changes, allowing leukocyte trafficking into the organ. On one hand, pro-inflammatory molecules, like TNF-α or IL-1α, which can be secreted by cancer cells, activate ECs. On the other, pro-angiogenic factors (VEGF, bFGF) were shown to reduce the amount of adhesion molecules on ECs (146). These dual effects are reflected in leukocyte trafficking through the tumor endothelium. It was shown that vessels present in the tumors lose P-selectin that limits the infiltration of leukocytes, making tumors inaccessible for the immune response (147). High VEGF levels were linked to lower levels of ICAM1 and T cell infiltration (145). This is affected by anti-cancer treatment, as ipilimumab plus bevacizumab could restore ICAM/VCAM expression on ECs, enhancing infiltration of cytotoxic lymphocytes (148). On the other hand, levels of E-selectin were increased in tumoral vessels in breast cancer and were also present in surrounding inflamed adipose tissue vasculature (149). Expression of this adhesion molecule allowed monocyte infiltration; however, these monocytes could be TAMs as their presence predicted poor survival. In pancreatic cancer, upregulation of adhesion proteins on the endothelium, including E-selectin, MAdCAM-1 and VCAM-1, allowed increased infiltration of Tregs (150). E-selectin was shown to favorably promote infiltration of non-protective Th2- polarized cells (151). Immune infiltrate can also be shaped due to the production of chemokines by tumor endothelial cells (TECs). ECs in the TME were characterized by downregulation of immuno-attractant molecules (CCL2, CCL18, IL-6) (152), additionally further limiting the leukocyte infiltration. At the same time, TECs were described to possess a specific secretory profile, including IL-4, -13, -6, -8, and TNF-α, which can modulate immune responses (153). Another way ECs

can affect immune cells is by directly interacting with them, for example through PD-L1. It was shown that endothelial PD-L1 is increased in several cancers in comparison to healthy tissue, which coincided with lower infiltration of T cells and dominance of Tregs (154).

Therefore, alteration of adhesion molecule patterns and levels of secreted factors in TECs can mediate selective infiltration of immunosuppressive leukocytes promoting tumor growth or make the tumor impenetrable for protective immune cells (so called "cold", uninflamed tumors). This points to the role of ECs as contributors to shaping the immunosuppressive TME.

Immune-Modulating Role of VEGF and Other Pro-Angiogenic Factors

Apart from the modulatory role on ECs, proangiogenic factors were shown to affect immune cells' function. It was observed that VEGF can directly expand Tregs, recruit MDSC and inhibit DC maturation (155). VEGFR is selectively present on Tregs and not effector T cells, which explains homing of immunosuppressive cells into proangiogenic TME (156). It is also a known factor promoting Th2 responses that are usually not protective in cancer. Additionally, VEGF induces expression of immunosuppressive PD-1 on T lymphocytes (157). Another proangiogenic factor with strong immunomodulatory potential is FGF (158). It was shown to polarize macrophages into M2 subtype (159) and expand MDSCs (160). Interestingly, anti-FGF treatment caused broader T-cell receptor repertoire, probably due to increased cancer cells apoptosis (161) that shows an additional aspect that can be altered by proangiogenic and immunomodulatory molecules. Similarly, however less studied, activities were reported for PDGF. This growth factor is an important regulator of angiogenesis, especially during development (162), and tends to increase during EMT in cancer cells (163). It can inhibit maturation of DCs and induce IL-10 producing T cells with regulatory phenotype (164). A strongly angiogenic chemokine, IL-8 (165), affects several immune cells, mostly by promoting their adhesion to the endothelium and subsequent migration towards inflamed tissue. However, it was observed that IL-8 mediates recruitment into the tumor of MDSCs and N2, pro-tumoral neutrophils (166), pointing to the potential immunomodulating action of this chemokine.

To sum up, angiogenic switch and consequently pathological angiogenesis on several levels affect immune response. As factors shaping TME, they contribute to induction of immunosuppression and/or allow the tumor to remain immunoevasive, both by not alleviating hypoxia and maintaining a pro-angiogenic and immunomodulatory milieu.

THE HIDDEN POTENTIAL OF HYPOXIA-MODULATED GENETIC HETEROGENEITY IN EVOKING AN IMMUNE RESPONSE

In the tumor microenvironment, hypoxia is often associated with genomic instability through downregulation of DNA repair processes and replication signaling mechanisms. Although the

DNA damage sensing and signaling mechanisms are on high-alert for recognition of plausible DNA damage under acute/chronic hypoxia, the DNA repair pathways such as homologous recombination, Non-Homologous End Joining (NHEJ) and mismatch repair, are downregulated (8). The effect of hypoxia on DNA repair pathways and related genes has been reviewed elsewhere (167–169). In contrast, chronic hypoxia/anoxia for longer durations can induce replication stress due to downregulation of ribonucleotide reductase and depletion of deoxyribonucleotides (170). The downregulation of these processes contributes to genetic heterogeneity in tumors through induction of chromosomal instability, point mutations, and genome-doubling events (8). Hypoxia induced structural changes (large deletions, copy number aberrations, duplications, and truncations) are substantially higher than single nucleotide alterations, according to a recent study examining the pan-cancer data sets (171). Hypoxia is associated with increased mutational load and hypoxia associated early mutations occur in key driver genes like *BCL2*, *TP53*, *MYC*, *PTEN* and *VHL* (171). Although driver mutations contribute to clonal development of tumors, branching mutations are the major cause of intratumor genetic heterogeneity and play a key role in drug resistance (172). In a clinical setting, irrespective of the tumor type, branched evolution remains the norm and influence the tumor's evolutionary trajectory (173). The contribution of hypoxia to branched evolution of tumors can be extrapolated from a study done by Gerlinger and coworkers analyzing the effect of *VHL* driver mutations in clear cell renal cell carcinomas (ccRCC). *VHL* mutations are seen in 80% of the ccRCC and contribute to constant pseudohypoxia phenotype (174, 175). Using multi-region sequencing and phylogenetic analysis, the study revealed that the inactivation of the *VHL* gene through mutations/methylation were a founder event in the trunk of the phylogenetic trees (176) and showed a heterogeneity in genomic landscape among the subclones with wide-ranging clinical outcome. *In vitro* experimental studies have shown that hypoxia induces a panoply of single nucleotide variations and contributes to microevolution of tumors. However, in a clinical setting, it is noted that chromosomal instability is a major contributor of tumors heterogeneity and a major determinant of clinical outcome in cancers (177). Hypoxia exerts selection pressure to accelerate the adaptation of more competent chromosomally unstable tumor clones in several ways (178). Hypoxia triggers the selection of mutant clones (for example, *TP53*-mutated tumors) by allowing them to evade apoptotic mechanisms (179). A hypoxic microenvironment promotes cell competition and metastases by HIF-1α mediated epithelial-mesenchymal transition (178). Hypoxia drives the immune-escape of tumors by inducing the expression of immune checkpoint inhibitors and controlling the antigen presenting mechanisms (180). Genome-doubling events associated with hypoxia have been found *in vitro* in melanoma cells with an increase in levels of tetraploid cells, however, such events in clinical samples are not well understood (181).

Inactivation of DNA repair pathways can lead to significant increase in tumor mutational burden (182, 183). The contribution of DNA repair and replication processes to genomic instability under hypoxic conditions is clearly evident from the defective homologous recombination and defective mismatch repair related mutational signatures (171). Single base substitution signatures and insertion and deletion signature analysis reveals that high-hypoxia is associated with clonal mutations in tumors rather than subclonal mutations (171). Furthermore, hypoxia is associated with increase in APOBEC activity and cyclic hypoxia induced replication stress provides single stranded DNA substrates for APOBEC mediated mutagenesis in breast, lung, and colorectal cancers (184).

Increased neoantigen load renders the tumor immunogenic with increased infiltration of lymphocytes leading to better clinical response to ICI in non-small cell lung cancer, melanoma, colorectal adenocarcinoma (185). In a breast cancer model, recent research from our group found that tumor hypoxia increased tumor mutational load and potential neoantigens (186). Using publicly available datasets, Bhandari and coworkers revealed that high-hypoxia is associated with increased TMB at pan-cancer level (171). However, clinical evidence on hypoxia-induced TMB and neoantigen burden is lacking. On the contrary, tumor hypoxia leads to an 'immune-cold' environment. Hypoxic tumor microenvironment is associated with immune evasion through expression of immune checkpoints (programmed death ligand -1), downregulation of type-I interferon signaling, shedding of antigen presenting molecules (MHC class I), enrichment of immunosuppressive cytokines and aggregation of immune suppressive cells (MDSC and Tregs) in the TME (187). In this regard, recent attempts to target hypoxic cells selectively with hypoxia activated prodrugs have yielded encouraging results with a significant antitumor response to immune checkpoint blockade. Jayaprakash and coworkers, using transgenic adenocarcinoma of the mouse prostate (TRAMP-C2), demonstrated that hypoxia targeting through Evofosfamide restored the T cell infiltration within the tumor and enhanced the response to immune checkpoint blockade (12). A study by Lequeux and coworkers investigated the inhibition of HIF-1α activity on cytotoxic immune cell infiltration into B16-F10 melanoma, and found an increase in infiltration of NK and CD8+ effector T cells and a significantly increased response to anti-PD-1 blockade (13). A comprehensive understanding of hypoxia induced mutational burden, neoantigen load will be crucial for enhancing the immunotherapy response in ICI resistant tumors.

UNRAVELING THE HYPOXIA-IMMUNE CONTEXTURE *IN SILICO*: THE HITS AND THE MISSES

The methods utilized thus far to study the immune contexture and degree of hypoxia in tumors have mainly done so separately. Regarding tumor infiltrating immune populations, imaging techniques including immunohistochemistry (IHC) and fluorescence microscopy, as well as cytometry-based methods,

using cell/population-specific antibodies have been the standard approach (188). For hypoxia, in addition to IHC which is used to check hypoxia induced proteins (like CAIX and GLUT1), various imaging techniques, such as positron emission tomography (PET), oxygen-enhanced (OE) magnetic resonance imaging (MRI), as well as blood oxygen level dependent (BOLD) and tissue oxygen level dependent (TOLD) MRI, have been utilized (9, 106). The focus of this section, however, is the uprise of *in silico* approaches to simultaneously navigate both the immune and hypoxic aspects of the TME.

In the last decade, the application of hypoxia gene signatures to reflect the degree of tumor hypoxia has taken the literature by storm with published signatures covering almost every solid tumor type (9, 106). In addition to that, there has been an escalating number of papers focused not only on designating the hypoxic state of a tumor, but also interrogating the immune populations and immune activation status of that tumor depending on its hypoxic phenotype (189–197). The process generally entails first deriving a hypoxia signature in the cancer type of interest, which often takes the route of narrowing down a list of hypoxia-related differentially expressed genes (DEGs) between normal and tumor tissue, then determining which genes are correlated with patient prognosis, be it overall survival or disease-free survival. The top genes and the factor by which they influence survival are then put together in a formula to calculate the risk score. Each sample is then allocated to the high-risk or low-risk group depending on their expression of the signature genes and whether their score is greater or less than the median risk score of the entire cohort. The score in this case is not only reflective of the hypoxic state of the tumor but is also associated with worse patient prognosis. An alternate strategy has also been used to group patients into high and low hypoxia groups based on their hypoxia score (197). The hypoxia score is calculated according to their expression levels of the hypoxia signature genes alone, without incorporating a risk parameter. Here again, the distribution is based on the variation from the median expression of the signature genes, and higher score is associated with worse survival. In either case, the next step has been to apply different tools or immune signatures to compare the two groups to make conclusions on the immune microenvironment in the context of hypoxia.

Several computational tools exist that rely on a tumor's bulk transcriptomic data to enumerate its existing immune populations (188, 198). These tools employ both a selected statistical framework as well as a base signature matrix or gene set representing the immune cell types of interest to deduce the tumor's respective immune phenotype (188, 199). The statistical framework is a variation of one of two primary algorithms, enrichment, or deconvolution. Gene set enrichment gives a semiquantitative score describing the enrichment of a cell type of interest in a sample based on the ranking of cell-type specific marker genes compared to all other genes present (198). A variation of that algorithm is single-sample GSEA (ssGSEA), in which the enrichment score is computed to represent the coordinately upregulated or downregulated genes within a single sample (198, 200). On the other hand, deconvolution

algorithms consider the transcriptome profile of a heterogenous sample as a linear mixture of gene expression levels of distinct cell types. The unknown cell fraction of interest can then be estimated by determining the weighted contribution of each gene to a signature matrix that includes the cell-type specific expression profiles (198). In this way, tools based on the deconvolution algorithm give quantitative estimates of relative cell fractions; however, given that in a heterogeneous sample cell types having higher amounts of total mRNA will have a stronger contribution to the mixture, such cell types may be overestimated (198).

An important determinant for the effective estimation of the immune cell populations is the quality and accuracy of the gene set or base signature matrix being incorporated by the computational tool (201, 202). For example, Estimation of STromal and Immune cells in MAlignant Tumours using Expression data (ESTIMATE) uses a gene set derived from the overlap between gene expression profiles (GEPs) of normal hematopoietic samples and genes associated with the quantity of immune cells infiltrating tumor tissue (203). This gene set constitutes the immune signature and is used to give a tumor sample an immune score. The tool also has a gene set representing the stromal signature and uses that to give the same sample a stromal score. The combination of the two scores indicates the ESTIMATE score, or tumor purity.

With respect to deconvolution-based tools, the first base signature matrices to be used were derived from microarray data conducted on FACS-derived subsets of cells originating from peripheral blood mononuclear cells (PBMCs) of healthy individuals, or *in vitro* stimulated and differentiated cells (204, 205). These result in suboptimal coverage of cellular phenotypes in complex tissues and prevent the discovery of possible new cellular states, as well as gene expression profiles that are cell-type specific (206). Furthermore, tools based on such base matrices, are only compatible with microarray derived gene expression profile of a tumor sample. Such tools, include Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts (CIBERSORT), which can be applied with a leukocyte signature matrix representing 22 immune cells to deconvolute and resolve the relative fractions of these cells in complex tissue (205). To overcome the stated limitations, an upgraded computational framework of CIBERSORT, termed CIBERSORTx, has been formulated using cell type-specific reference profiles derived from single cell RNA sequencing, allowing cross-platform normalization and *in silico* cell purification (206). Starting form RNA expression profiles of intact whole tissue samples, the cell-type-specific GEPs and abundance of each cell type can then be accurately inferred (206). Indeed, this tool is heralded as a digital cytometer that negates the need for physical dissociation, living material or antibodies, yet manages to give a detailed portrait of tissue components from bulk RNA admixtures (206).

In addition to ESTIMATE, CIBERSORT and CIBERSORTx, other commonly used tools include Microenvironment Cell Populations (MCP)-counter, which computes an abundance estimate of eight different immune cell types and two stromal cells (fibroblasts and endothelial cells) (207); as well as the

webtool Tumor IMmune Estimation Resource (TIMER), which provides the proportions of six immune cells, CD4+ and CD8+ T cells, B cells, DCs, macrophages, and neutrophils in the tissue of 23 tumor types from The Cancer Genome Atlas (TCGA) (208). One more tool of interest is ImmuCellAI (Immune Cell Abundance Identifier) that uses signatures to give abundance estimates on 24 immune cells including 18 subtypes of T cells, as well as B cells, DCs, monocytes, NK cells, macrophages, and neutrophils. Both RNA-Seq and microarray expression data are compatible with this tool (209). A final tool worth mentioning here is TIDE, which stands for Tumor Immune Dysfunction and Exclusion. This tool goes beyond determining the immune status of a tumor and potential immune escape. It was developed to use gene expression profiles of cancer samples to predict response to ICI by reporting on both immune and stromal cellular elements, a tissue agnostic interferon gamma signature, as well as the enrichment scores of ICI biomarkers, microsatellite instability and PD-L1, among others (210).

Table 1 represents select studies that have used one or a combination of the computational approaches to annotate the tumor immune microenvironment and merged that with a hypoxia gene signature to distinguish the more hypoxic from the less hypoxic tumors. As evident from the table, every signature consists of its own set of genes, even if it was derived from the same tumor type, with minimal overlap with other signatures. The conclusions of most papers underscore the immunosuppressive power of hypoxia in bladder cancer (189, 213), breast cancer (214), colorectal cancer (CRC) (191, 216, 217), head and neck squamous cell carcinoma (218), hepatocellular carcinoma (HCC) (221–223), lung cancer (195, 225, 226), melanoma (196), oral squamous cell carcinoma (227), osteosarcoma (229), ovarian carcinoma (230) and pancreatic ductal adenocarcinoma (PDAC) (197, 231). In the case of bladder cancer, two other studies determined the presence of high infiltration of immune cells in the high-risk group and found positive correlations between immune score and the risk score (190, 212). The authors also showed that despite the presence of both tumor-promoting and tumor-antagonizing immune cells, the risk score was positively correlated with immune checkpoints. One study even went on to report a potentially enhanced response of the high-risk group to immunotherapy (190). With respect to CRC, the only contradictory study included a single cohort and only focused on GSEA and the ESTIMATE score, not considering immune populations (215). In terms of HCC, one study reported a higher immune score in the high-risk group (219) as well as a significant infiltration of immune cells in this group which also showed enhanced predicted response to ICI (220). In a study that integrated 11 independent HCC cohorts, not a single immune cell population could be significantly differentiated in a consistent manner between the low-risk and high-risk groups, highlighting the complexity of the factors determining immune cell infiltration (194). It is difficult to make any conclusion on the findings in renal cell carcinoma, as all three studies included a single cohort respectively and reported distinct findings (232–234) (**Table 1**).

It is clear that to resolve the discrepancies identified for even the same tumor type, a validated hypoxia gene signature should be utilized to score the tumor and the *in silico* analysis of the immune contexture should be done using signatures that reflect the immune state of the specific cancer type. The tools utilized thus far apply gene expression profiles obtained from immune cells of healthy PBMCs or tissue, meanwhile based on the complexity of mechanisms governing the activation state of immune cells, a cancer-specific approach would provide a more accurate representation of the immune contexture. This would ultimately enhance the quality of the findings being generated from these tools, making them more consistent and having higher accuracy. Furthermore, one downside remains with the inability of such tools to give information about the localization of the reported immune populations in the tumor mass. This spatial dimension is intercalated with the global functional state of the immune response and is now being investigated with advanced techniques, including single cell transcriptomics using slide sequencing (235), as well as multiplex immunofluorescence imaging using the CODEX® System (236). Therefore, despite the simplicity and ease of use of current computational tools, they should not be used as a standalone analysis platform to conclude on the immune activation state of a tumor.

DISCUSSION

To date, clinical benefit from cancer immunotherapy has been limited to a minority of patients. Achieving benefit in the majority of patients necessitates a wholistic understanding of anti-tumor response mechanisms and both the cell-intrinsic and extrinsic molecules involved in primary, adaptive, as well as acquired resistance to immunotherapy. In this regard, it has become clear that the TME is likely to play a crucial role in cancer response to treatment. In fact, the growth and progression of cancer cells depend not only on their malignant potential, but also on the multidirectional interactions of cellular and metabolic components of tumor microenvironment. It is widely admitted that novel and continuously evolving pathological entities arise as a result of the interactions among tumor cells and stromal cells during cancer progression.

As previously reported, many cellular, molecular, and metabolic elements of the TME are emerging as attractive targets for therapeutic approaches (47, 59, 115, 122). In this respect, the existence of hypoxia in solid tumors is associated, not only with tumor invasion and metastasis, but also with a heightened risk of treatment failure and patient mortality and is currently attracting significant interest. Accumulating evidence indicates that hypoxia plays a key role in promoting the acquisition of tumor resistance to various antitumor immune effectors (26). Tumor hypoxia allows tumor cells to escape CTL- and NK -mediated killing through in part the activation of autophagy (97, 237, 238) and modulates the composition and function of the immune infiltrate (97, 237, 238). Hypoxic zones in tumors have also been reported to attract immunosuppressive cells such as MDSCs, tumor-associated macrophages and regulatory T cells. In addition, the association of hypoxia with cancer stemness in the tumor microenvironment of different cancer types is widely

TABLE 1 | Studies applying computational tools to investigate the immune landscape of tumors classified based on hypoxia signatures.

s.n	Cancer	Hypoxia Signature	Cohort Number[‡]	Immune Investigation Method	High-Risk Group (Hypoxia-High)	Low-Risk Group (Hypoxia-Low)	Reference
1	ACC*	3 genes (*CCNA2, COL5A1, EFNA3*)	1	CIBERSORT	Resting NK cell	Activated NK cell	(211)
2	BLCA	8 genes (*AKAP12, ALDOB, CASP6, DTNA, HS3ST1, JUN, KDELR3, STC1*)	3	CIBERSORT	M0 and M1 macrophages		(189)
3	BLCA[#]	4 genes (*ANXA2, COL5A1, GALK1, HS3ST1*)	1	ESTIMATE; ssGSEA	Immune and stromal scores positively correlated with risk score. Activated CD4 T cell, activated CD8 T cell, central memory CD8 T cell, effector memory CD8 T cell, gamma delta T cell, follicular helper T cell, Th1, Th2, aDC, pDC, activated B cell, immature B cell, memory B cell, NK cell, NK T cell, Treg, macrophage, MDSC, mast cell, monocyte, neutrophil, eosinophil	Th17, CD56bright NK cell	(212)
4	BLCA[#]	16 genes (*AKAP12, ANKZF1, CASP6, CCNG2, GALK1, GAPDH, HDLBP, HEXA, HS3ST1, SDC4, SLC2A1, SLC2A3, SRPX, STC1, VEGFA, WISP2*)	2	ssGSEA	CD8 T cell, NK cell, DC, Th1. Risk score positively related to T cell inflamed score and enrichment scores of immunotherapy-positive gene signatures		(190)
5	BLCA[#]	7 genes (*ALDOB, EGFR, FOXO3, GPC1, SDC4, SLC2A3, VEGFA*)	1	CIBERSORT	Resting mast cell, neutrophil, resting CD4 memory T cell	Follicular helper T cell, CD8 T cell, plasma cell	(213)
6	BC	13 genes (*ADM, ALDOA, CDKN3, LDHA, MIF, MRPS17, NDRG1, P4HA1, PGAM1, SLC2A1, TPI1, TUBB6, VEGFA*)	1	ImmuCellAI	nTreg cell, iTreg cell	CD8 T cell, CD4 T cell	(214)
7	CRC	5 genes (*ARL4C, CARS2, PSMD12, PTTG1IP, SEC61G*)	1	GSEA; ESTIMATE	Enriched immune pathways. Positively correlated with immune score and stromal score		(215)
8	CRC	12 genes (*CASP6, CYB5R3, DTX3L, FAM117B, IRF1, MBTD1, MINPP1, ORAI3, TNFAIP8, TRAF3, PRELID2, ZBTB44*)	2	ESTIMATE; CIBERSORT	Treg, M2 macrophage	Higher immune and stromal scores; CD4 T cell, M1 macrophage	(216)
9	CRC	4 genes (*ALDOB, ALDOC, GPC1, SLC2A3*)	2	CIBERSORT	M0 macrophage		(217)
10	CRC	356 genes	4	CIBERSORT	M0 and M2 macrophages	CD8 T cell, resting NK, resting CD4 memory T cell	(191)
11	GC[#]	2 genes (*EFNA3, SERPINE1*)	2	ESTIMATE; ssGSEA	Higher immune and stromal scores; Treg, macrophage, neutrophil, mast cell		(192)
12	Glioma[#]	5 genes (*GAPDH, HK2, JUN, LDHA, VEGFA*)	2	CIBERSORT	Resting CD4 memory T cell, Treg, resting NK cell, M0 macrophage, neutrophil		(193)
13	HNSCC	24 genes (*AMPD3, BHLHE40, COL5A1, CP, CSRP2, CXCR4, DDIT4, DUSP1, ERRFI1, F3, GPC4, HS3ST1, IL6, ISG20, MAFF, PGM2, PIM1, PLAC8, PPP1R3C, S100A4, SDC2, SELENBP1, SERPINE1, SRPX*)	1	CIBERSORT	Activated DC, M0 macrophage, eosinophil, activated mast cell, resting NK cell, resting CD4 memory T cell	Memory B cell, CD8 T cell, resting mast cell, Treg, follicular helper T cell, activated CD4 memory T cell, gamma delta T cell, plasma cell, activated NK cell	(218)
14	HCC	4 genes (*ENO1, GAPDH, LDHA, SLC2A1*)	1	ESTIMATE CIBERSORT	Higher immune score; Treg, M0 macrophage, neutrophil	Activated NK cell, M1 macrophage, resting mast cell	(219)
15	HCC[#]	24 genes (*ACOT7, ADM, ALDOA, ANGPTL4, BNC1, CA9, CDKN3, COL4A6, ENO1, FOSL1, GNAI1, LDHA, MIF, MRPS17, NDRG1, P4HA1, PGAM1, PGK1, SDC1, SLC16A1,*	1 meta-cohort (from 3 datasets)	MCP-counter; ssGSEA; TIDE	CD4 T cell, activated CD8 T cell, iDC, aDC, CD56bright and CD56dim NK cell, gamma delta T cell, immature B cell, macrophage, mast cell, MDSC, NK T cell, pDC, follicular helper T cell, Th1, Th2; Three times higher response to ICI	Eosinophil, neutrophil, Treg, Th17	(220)

(Continued)

TABLE 1 | Continued

s.n	Cancer	Hypoxia Signature	Cohort Number[‡]	Immune Investigation Method	High-Risk Group (Hypoxia-High)	Low-Risk Group (Hypoxia-Low)	Reference
		SLC2A1, TPI1, TUBB6, VEGFA)					
16	HCC[#]	21 genes (*ADM, BNIP3, BNIP3L, CA9, EGLN3, GDF15, GYS1, HCAR3, HILPDA, HK2, INSIG2, JUN, KDM3A, PFKFB4, PLIN2, PTPRH, SLC2A3, SMAD3, SPAG4, TMEM45A, WSB1*)	11	ESTIMATE; CIBERSORT	3 cohorts: higher stromal score; 6 cohorts: higher immune score; 6 cohorts: activated CD4 memory T cell; 5 cohorts: activated mast cell; 5 cohorts: M0 macrophage	6 cohorts: resting CD4 memory T cell; 5 cohorts: resting mast cell; 3 cohorts: NK cell	(194)
17	HCC[#]	3 genes (*CDCA8, PDSS1, SLC7A11*)	1	CIBERSORT	M0 macrophage, memory B cell, follicular helper T cell		(221)
18	HCC	10 genes (*APEX1, ATR, CTSA, DNAJC5, ENO1, EPO, HMOX1, LDHA, NDRG1, PER1*)	1	CIBERSORT	M0 macrophage, Treg, neutrophil, eosinophil	Resting mast cell, resting CD4 memory cell, M1 macrophage, monocyte	(222)
19	HCC	4 genes (*DCN, DDIT4, NDRG1, PRKCA*)	2	ssGSEA		Activated B cell, activated CD8 T cell, effector memory CD8 T cell, Treg, Th1, CD56bright NK cell, NK cell, NK T cell, eosinophil, macrophage, mast cell, MDSC, monocyte, pDC	(223)
20	NSCLC*	11 genes (*AMPD3, DDX11, FANCI, HIF-3α, IDE, LRP8, NOLC1, PAIP1, PDCD2, PSMF1, SNAPC5*)	1	ESTIMATE; ssGSEA		Higher immune score; DCs, aDCs, iDCs, pDCs, HLA, B cell, mast cell, neutrophil, T helper cell, T cell co-inhibition, T cell co-stimulation, TILs, Type II IFN response	(224)
21	NSCLC	4 genes (*ANGPTL4, PFKP, SLC2A1, XPNPEP1*)	2	CIBERSORT	Activated CD4 memory T cell, resting NK cell, M0 and M1 macrophages	Memory B cell, resting CD4 memory T cell, monocyte, resting DC, resting mast cell	(195)
22	NSCLC	18 genes (*ADM, BIK, DDIT3, ENO1, EPAS1, FGF3, GAPDH, MIF, NFKB1, PFKP, PGK1, PLAUR, SPP1, STC1, TEK, TFRC, TGFA, XRCC6*)	1	ssGSEA	Activated CD4 T cell, CD56bright NK cell, memory B cell, Th2	Activated B cell, activated CD8 T cell, central memory CD4 T cell, effector memory CD8 T cell, eosinophil, immature B cell, iDC, pDCs, macrophage, mast cell, MDSC, monocyte, NK cell, neutrophil, follicular helper T cell, Th1, Th17	(225)
23	NSCLC[#]	7 lncRNAs (AC010980.2, AC022784.1, AC079949.2, AC090001.1, AL161431.1, LINC00707, LINC00941)	1	CIBERSORT	Neutrophil, M0 and M2 macrophages	Monocyte	(226)
24	SKCM	11 genes (*CP, DPYSL4, EGFR, FBP1, FOXO3, IGFBP1, ISG20, KIF5A, PPARGC1A, S100A4, SDC3*)	2	CIBERSORT	Treg, mast cell; 1 cohort: resting CD4 memory T cell, monocyte	Activated CD4 memory T cell, M1 macrophage; 1 cohort: CD8 T cell, plasma cell	(196)
25	OSCC[#]	4 genes (*ALDOA, P4HA1, PGK1, VEGFA*)	1	CIBERSORT	M0 macrophage, mast cell	Naïve B cell, CD8 T cell, follicular helper T cell, Treg, neutrophil	(227)
26	OS*	2 genes (*P4HA1, DCN*)	2	ssGSEA		DC, pDC, macrophage, neutrophil, TIL	(228)
27	OS	4 genes (*EFNA1, P4HA1, STC2, MAFF*)	2	CIBERSORT	Resting CD4 memory T cell		(229)
28	OVC[#]	9 genes (*ALOX5AP, ANXA1, IGFBP2, LAG3, PLK3, SLC1A1, SREBF1, SREBF2, TGFB1*)	1	CIBERSORT; TIMER	Activated CD4 memory T cell, gamma-delta T cell, activated NK, neutrophil, M1 and M2 macrophages	Resting CD4 memory T cell, follicular helper T cell, Treg, aDC, resting mast cell; Higher MHC and antigen presenting molecules	(230)
29	PDAC[#]	8 genes (*DDIT4, LDHA, MXI1, NDRG1, P4HA1, PGK1, SLC2A1, VEGFA*)	2	CIBERSORTx	M0 macrophage	CD8 T cell; Higher immune score and cytolytic index[§]	(197)

(Continued)

TABLE 1 | Continued

s.n	Cancer	Hypoxia Signature	Cohort Number[‡]	Immune Investigation Method	High-Risk Group (Hypoxia-High)	Low-Risk Group (Hypoxia-Low)	Reference
30	PDAC	4 genes (*ENO3*, *LDHA*, *PGK1*, *PGM1*)	2	CIBERSORT	M2 macrophage, resting NK cell	CD8 T cell, naive B cell	(231)
31	RCC	9 lnRNA (AC002070.1, AC008760.2, AC084876.1, AC147651.1, FOXD2-AS1, ITPR1-DT, LINC00944, LINC01615, LINC02027)	1	CIBERSORT	Plasma cell, follicular helper T cell, Treg	M2 macrophage, resting DC, resting mast cell	(232)
32	RCC	4 lnRNA (AC026462.3, COMETT, EMX2OS, HAGLR)	1	TIMER; ESTIMATE	B cell, CD4 T cell, CD8 T cell, DC, macrophage, neutrophil positively correlated with risk score. Higher immune and stromal scores		(233)
33	RCC[#]	8 genes (*BCL2*, *KDELR3*, *KLF6*, *PCK1*, *PLAUR*, *PPARGC1A*, *RORA*, *WSB1*)	1	CIBERSORT	Treg, CD8 T cell, follicular helper T cell, plasma cell, M0 macrophage, activated NK cell	Resting CD4 memory, monocyte, M1 macrophage, resting mast cell, resting NK cell	(234)

[‡]*Number of independent patient cohorts analyzed with indicated method to investigate immune tumor microenvironment.*
[#]*Studies reporting higher immune checkpoint inhibitors or immunosuppressive cytokines or both in High-risk group.*
[*]*Studies reporting higher immune checkpoint inhibitors or immunosuppressive cytokines or both in Low-risk group.*
[§]*Immune score calculated based on an eighteen gene tumor inflammation signature. The cytolytic index calculated based on the geometric mean of the GZMA (granzyme A) and PRF1 (perforin-1) produced by activated cytolytic CD8+ T cells (197).*
s.n, serial number; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BC, breast cancer; CRC, colorectal cancer; GC, gastric cancer; HNSCC, head and neck squamous cell carcinoma; HCC, hepatocellular carcinoma; NSCLC, non-small cell lung cancer; SKCM, skin cutaneous melanoma; OSCC, oral squamous cell carcinoma; OS, osteosarcoma; OVC, ovarian carcinoma; PDAC, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma; lnRNA, long non-coding RNA; CIBERSORT, Cell-type Identification By Estimating Relative Subsets Of RNA Transcripts; ESTIMATE, Estimation of STromal and Immune cells in MAlignant Tumours using Expression data; GSEA, gene set enrichment analysis; ssGSEA, single-sample GSEA; MCP-counter, Microenvironment Cell Populations-counter; TIMER, Tumor IMmune Estimation Resource; ImmuCellAI, Immune Cell Abundance Identifier; DC, dendritic cell; aDC, activated DC; iDC, immature DC; pDC, plasmacytoid DC; Th1, type 1 T helper cell; Th2, type 2 T helper cell; Th17, T helper 17 cell; Treg, regulatory T cell; iTreg, induced Treg; nTreg, natural Treg; HLA, human leukocyte antigen; TIL, tumor infiltrating leukocytes; IFN, interferon; MHC, major histocompatibility complex.

admitted. Therefore, controlling hypoxic stress to avoid tumor resistance and to reshape the hypoxic immunosuppressive TME in order to improve cancer immunotherapy remains a relevant challenge. Developing pharmacological agents to modulate HIF-1α signaling pathway is still attracting significant interest in the field of oncoimmunology. Several sub-types of drugs have been reported to inhibit HIF-1α activity including inhibitors of HIF-1α/HIF-1β dimerization (for example, acriflavine) (239, 240). Very recently, we demonstrated that suppression of the transcriptional activity of HIF-1α resulted in an increased infiltration of NK cells and CD8+ T cells in the tumor microenvironment of melanoma (13). Hypoxia could therefore be a potential immunometabolic checkpoint with prognostic value by regulating the TME and affecting the interaction between tumor cells and immune cells.

It is now well established that high expression of clonal tumor neoantigens correlates with an upregulation of lymphocyte infiltration within a tumor, enhanced patient survival and a prolonged response to immunotherapy. Recently, others and we have demonstrated that hypoxia interferes with genetic instability by inducing DNA damages, inducing DNA repair alteration (186) and presumably the emergence of tumor neoantigens. While the main predictive biomarkers for immunotherapy involve microsatellite instability/defective mismatch repair (MSI/dMMR), and tumor mutational burden, based on our previous reports and those of other teams, tumor hypoxia should be also be exploited as a potential biomarker to predict immunotherapy outcomes.

A deeper understanding of the role of hypoxia in killer cell induction and migration, immune suppression and EMT could enable the creation of more highly refined, innovative and integrative immunotherapies, targeting tumor plasticity and heterogeneity and aiding in overcoming the inherent constraints of currently applied anticancer therapies. In addition to the known hypoxic signatures reported, we believe that the design of minimally- or even non-invasive techniques able to predict treatment efficacy and tumor recurrence through algorithm-based modeling of network dynamics and by generating models based on artificial intelligence, or through the integration of "omics", must be considered in the field of oncoimmunology.

AUTHOR CONTRIBUTIONS

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REFERENCES

1. Galon J, Bruni D. Tumor Immunology and Tumor Evolution: Intertwined Histories. *Immunity* (2020) 52(1):55–81. doi: 10.1016/j.immuni.2019.12.018

2. Chouaib S, Umansky V, Kieda C. The Role of Hypoxia in Shaping the Recruitment of Proangiogenic and Immunosuppressive Cells in the Tumor Microenvironment. *Contemp Oncol (Pozn)* (2018) 22(1A):7–13. doi: 10.5114/wo.2018.73874

3. You L, Wu W, Wang X, Fang L, Adam V, Nepovimova E, et al. The Role of Hypoxia-Inducible Factor 1 in Tumor Immune Evasion. *Med Res Rev* (2021) 41(3):1622–43. doi: 10.1002/med.21771

4. Semenza GL. Intratumoral Hypoxia and Mechanisms of Immune Evasion Mediated by Hypoxia-Inducible Factors. *Physiol (Bethesda)* (2021) 36 (2):73–83. doi: 10.1152/physiol.00034.2020

5. Samanta D, Semenza GL. Metabolic Adaptation of Cancer and Immune Cells Mediated by Hypoxia-Inducible Factors. *Biochim Biophys Acta Rev Cancer* (2018) 1870(1):15–22. doi: 10.1016/j.bbcan.2018.07.002

6. Zhang H, Bosch-Marce M, Shimoda LA, Tan YS, Baek JH, Wesley JB, et al. Mitochondrial Autophagy Is an HIF-1-Dependent Adaptive Metabolic Response to Hypoxia. *J Biol Chem* (2008) 283(16):10892–903. doi: 10.1074/jbc.M800102200

7. Lequeux A, Noman MZ, Xiao M, Sauvage D, Van Moer K, Viry E, et al. Impact of Hypoxic Tumor Microenvironment and Tumor Cell Plasticity on the Expression of Immune Checkpoints. *Cancer Lett* (2019) 458:13–20. doi: 10.1016/j.canlet.2019.05.021

8. Tang M, Bolderson E, O'Byrne KJ, Richard DJ. Tumor Hypoxia Drives Genomic Instability. *Front Cell Dev Biol* (2021) 9:626229. doi: 10.3389/fcell.2021.626229

9. Abou Khouzam R, Brodaczewska K, Filipiak A, Zeinelabdin NA, Buart S, Szczylik C, et al. Tumor Hypoxia Regulates Immune Escape/Invasion: Influence on Angiogenesis and Potential Impact of Hypoxic Biomarkers on Cancer Therapies. *Front Immunol* (2020) 11:613114. doi: 10.3389/fimmu.2020.613114

10. Semenza GL. Defining the Role of Hypoxia-Inducible Factor 1 in Cancer Biology and Therapeutics. *Oncogene* (2010) 29(5):625–34. doi: 10.1038/onc.2009.441

11. Noman MZ, Hasmim M, Lequeux A, Xiao M, Duhem C, Chouaib S, et al. Improving Cancer Immunotherapy by Targeting the Hypoxic Tumor Microenvironment: New Opportunities and Challenges. *Cells* (2019) 8 (9):1083. doi: 10.3390/cells8091083

12. Jayaprakash P, Ai M, Liu A, Budhani P, Bartkowiak T, Sheng J, et al. Targeted Hypoxia Reduction Restores T Cell Infiltration and Sensitizes Prostate Cancer to Immunotherapy. *J Clin Invest* (2018) 128(11):5137–49. doi: 10.1172/JCI96268

13. Lequeux A, Noman MZ, Xiao M, Van Moer K, Hasmim M, Benoit A, et al. Targeting HIF-1 Alpha Transcriptional Activity Drives Cytotoxic Immune Effector Cells Into Melanoma and Improves Combination Immunotherapy. *Oncogene* (2021) 40(28):4725–35. doi: 10.1038/s41388-021-01846-x

14. Gropper Y, Feferman T, Shalit T, Salame TM, Porat Z, Shakhar G. Culturing CTLs Under Hypoxic Conditions Enhances Their Cytolysis and Improves Their Anti-Tumor Function. *Cell Rep* (2017) 20(11):2547–55. doi: 10.1016/j.celrep.2017.08.071

15. Palazon A, Tyrakis PA, Macias D, Velica P, Rundqvist H, Fitzpatrick S, et al. An HIF-1alpha/VEGF-A Axis in Cytotoxic T Cells Regulates Tumor Progression. *Cancer Cell* (2017) 32(5):669–83 e5. doi: 10.1016/j.ccell.2017.10.003

16. Cho SH, Raybuck AL, Blagih J, Kemboi E, Haase VH, Jones RG, et al. Hypoxia-Inducible Factors in CD4(+) T Cells Promote Metabolism, Switch Cytokine Secretion, and T Cell Help in Humoral Immunity. *Proc Natl Acad Sci USA* (2019) 116(18):8975–84. doi: 10.1073/pnas.1811702116

17. Hayakawa Y, Kelly JM, Westwood JA, Darcy PK, Diefenbach A, Raulet D, et al. Cutting Edge: Tumor Rejection Mediated by NKG2D Receptor-Ligand Interaction Is Dependent Upon Perforin. *J Immunol* (2002) 169(10):5377–81. doi: 10.4049/jimmunol.169.10.5377

18. Cheon H, Borden EC, Stark GR. Interferons and Their Stimulated Genes in the Tumor Microenvironment. *Semin Oncol* (2014) 41(2):156–73. doi: 10.1053/j.seminoncol.2014.02.002

19. Rouette A, Trofimov A, Haberl D, Boucher G, Lavallée VP, D'Angelo G, et al. Expression of Immunoproteasome Genes Is Regulated by Cell-Intrinsic

and -Extrinsic Factors in Human Cancers. *Sci Rep* (2016) 6:34019. doi: 10.1038/srep34019

20. Aras S, Zaidi MR. TAMeless Traitors: Macrophages in Cancer Progression and Metastasis. *Br J Cancer* (2017) 117(11):1583–91. doi: 10.1038/bjc.2017.356

21. Orecchioni M, Ghosheh Y, Pramod AB, Ley K. Macrophage Polarization: Different Gene Signatures in M1(LPS+) vs. Classically and M2(LPS-) vs. Alternatively Activated Macrophages. *Front Immunol* (2019) 10:1084. doi: 10.3389/fimmu.2019.01084

22. Chen DS, Mellman I. Oncology Meets Immunology: The Cancer-Immunity Cycle. *Immunity* (2013) 39(1):1–10. doi: 10.1016/j.immuni.2013.07.012

23. Gardner A, Ruffell B. Dendritic Cells and Cancer Immunity. *Trends Immunol* (2016) 37(12):855–65. doi: 10.1016/j.it.2016.09.006

24. Leone RD, Powell JD. Metabolism of Immune Cells in Cancer. *Nat Rev Cancer* (2020) 20(9):516–31. doi: 10.1038/s41568-020-0273-y

25. Chiche J, Brahimi-Horn MC, Pouysségur J. Tumour Hypoxia Induces a Metabolic Shift Causing Acidosis: A Common Feature in Cancer. *J Cell Mol Med* (2010) 14(4):771–94. doi: 10.1111/j.1582-4934.2009.00994.x

26. Chouaib S, Noman MZ, Kosmatopoulos K, Curran MA. Hypoxic Stress: Obstacles and Opportunities for Innovative Immunotherapy of Cancer. *Oncogene* (2017) 36(4):439–45. doi: 10.1038/onc.2016.225

27. Pietrobon V, Marincola FM. Hypoxia and the Phenomenon of Immune Exclusion. *J Transl Med* (2021) 19(1):9. doi: 10.1186/s12967-020-02667-4

28. Chang CH, Curtis JD, Maggi LB, Faubert B, Villarino AV, O'Sullivan D, et al. Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. *Cell* (2013) 153(6):1239–51. doi: 10.1016/j.cell.2013.05.016

29. Chang CH, Qiu J, O'Sullivan D, Buck MD, Noguchi T, Curtis JD, et al. Metabolic Competition in the Tumor Microenvironment Is a Driver of Cancer Progression. *Cell* (2015) 162(6):1229–41. doi: 10.1016/j.cell.2015.08.016

30. Sheppard S, Santosa EK, Lau CM, Violante S, Giovanelli P, Kim H, et al. Lactate Dehydrogenase A-Dependent Aerobic Glycolysis Promotes Natural Killer Cell Anti-Viral and Anti-Tumor Function. *Cell Rep* (2021) 35 (9):109210. doi: 10.1016/j.celrep.2021.109210

31. Angelin A, Gil-de-Gómez L, Dahiya S, Jiao J, Guo L, Levine MH, et al. Foxp3 Reprograms T Cell Metabolism to Function in Low-Glucose, High-Lactate Environments. *Cell Metab* (2017) 25(6):1282–93.e7. doi: 10.1016/j.cmet.2016.12.018

32. 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(3):1486–95. doi: 10.4049/jimmunol.1202702

33. Brand A, Singer K, Koehl GE, Kolitzus M, Schoenhammer G, Thiel A, et al. LDHA-Associated Lactic Acid Production Blunts Tumor Immunosurveillance by T and NK Cells. *Cell Metab* (2016) 24(5):657–71. doi: 10.1016/j.cmet.2016.08.011

34. Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, Andreesen R, et al. Tumor-Derived Lactic Acid Modulates Dendritic Cell Activation and Antigen Expression. *Blood* (2006) 107(5):2013–21. doi: 10.1182/blood-2005-05-1795

35. Comito G, Iscaro A, Bacci M, Morandi A, Ippolito L, Parri M, et al. Lactate Modulates CD4. *Oncogene* (2019) 38(19):3681–95. doi: 10.1038/s41388-019-0688-7

36. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional Polarization of Tumour-Associated Macrophages by Tumour-Derived Lactic Acid. *Nature* (2014) 513(7519):559–63. doi: 10.1038/nature13490

37. Shan T, Chen S, Chen X, Wu T, Yang Y, Li S, et al. M2−TAM Subsets Altered by Lactic Acid Promote T−Cell Apoptosis Through the PD−L1/PD−1 Pathway. *Oncol Rep* (2020) 44(5):1885–94. doi: 10.3892/or.2020.7767

38. Hao X, Ren Y, Feng M, Wang Q, Wang Y. Metabolic Reprogramming Due to Hypoxia in Pancreatic Cancer: Implications for Tumor Formation, Immunity, and More. *BioMed Pharmacother* (2021) 141:111798. doi: 10.1016/j.biopha.2021.111798

39. Klysz D, Tai X, Robert PA, Craveiro M, Cretenet G, Oburoglu L, et al. Glutamine-Dependent α-Ketoglutarate Production Regulates the Balance Between T Helper 1 Cell and Regulatory T Cell Generation. *Sci Signal* (2015) 8(396):ra97. doi: 10.1126/scisignal.aab2610

40. Leone RD, Zhao L, Englert JM, Sun IM, Oh MH, Sun IH, et al. Glutamine Blockade Induces Divergent Metabolic Programs to Overcome Tumor Immune Evasion. *Science* (2019) 366(6468):1013–21. doi: 10.1126/science.aav2588

41. Ye LY, Chen W, Bai XL, Xu XY, Zhang Q, Xia XF, et al. Hypoxia-Induced Epithelial-To-Mesenchymal Transition in Hepatocellular Carcinoma Induces an Immunosuppressive Tumor Microenvironment to Promote Metastasis. *Cancer Res* (2016) 76(4):818–30. doi: 10.1158/0008-5472.CAN-15-0977

42. Munn DH, Sharma MD, Baban B, Harding HP, Zhang Y, Ron D, et al. GCN2 Kinase in T Cells Mediates Proliferative Arrest and Anergy Induction in Response to Indoleamine 2,3-Dioxygenase. *Immunity* (2005) 22(5):633–42. doi: 10.1016/j.immuni.2005.03.013

43. Song X, Zhang Y, Zhang L, Song W, Shi L. Hypoxia Enhances Indoleamine 2,3-Dioxygenase Production in Dendritic Cells. *Oncotarget* (2018) 9(14):11572–80. doi: 10.18632/oncotarget.24098

44. Liu J, Zhang H, Jia L, Sun H. Effects of Treg Cells and IDO on Human Epithelial Ovarian Cancer Cells Under Hypoxic Conditions. *Mol Med Rep* (2015) 11(3):1708–14. doi: 10.3892/mmr.2014.2893

45. Schmidt SK, Ebel S, Keil E, Woite C, Ernst JF, Benzin AE, et al. Regulation of IDO Activity by Oxygen Supply: Inhibitory Effects on Antimicrobial and Immunoregulatory Functions. *PLoS One* (2013) 8(5):e6301:3. doi: 10.1371/journal.pone.0063301

46. Mohapatra SR, Sadik A, Tykocinski LO, Dietze J, Poschet G, Heiland I, et al. Hypoxia Inducible Factor 1α Inhibits the Expression of Immunosuppressive Tryptophan-2,3-Dioxygenase in Glioblastoma. *Front Immunol* (2019) 10:2762. doi: 10.3389/fimmu.2019.02762

47. Labadie BW, Bao R, Luke JJ. Reimagining IDO Pathway Inhibition in Cancer Immunotherapy *via* Downstream Focus on the Tryptophan-Kynurenine-Aryl Hydrocarbon Axis. *Clin Cancer Res* (2019) 25(5):1462–71. doi: 10.1158/1078-0432.CCR-18-2882

48. Geiger R, Rieckmann JC, Wolf T, Basso C, Feng Y, Fuhrer T, et al. L-Arginine Modulates T Cell Metabolism and Enhances Survival and Anti-Tumor Activity. *Cell* (2016) 167(3):829–42.e13. doi: 10.1016/j.cell.2016.09.031

49. Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, et al. HIF-1α Regulates Function and Differentiation of Myeloid-Derived Suppressor Cells in the Tumor Microenvironment. *J Exp Med* (2010) 207(11):2439–53. doi: 10.1084/jem.20100587

50. Rodriguez PC, Quiceno DG, Ochoa AC. L-Arginine Availability Regulates T-Lymphocyte Cell-Cycle Progression. *Blood* (2007) 109(4):1568–73. doi: 10.1182/blood-2006-06-031856

51. Doedens AL, Stockmann C, Rubinstein MP, Liao D, Zhang N, DeNardo DG, et al. Macrophage Expression of Hypoxia-Inducible Factor-1 Alpha Suppresses T-Cell Function and Promotes Tumor Progression. *Cancer Res* (2010) 70(19):7465–75. doi: 10.1158/0008-5472.CAN-10-1439

52. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, et al. Arginase I in Myeloid Suppressor Cells Is Induced by COX-2 in Lung Carcinoma. *J Exp Med* (2005) 202(7):931–9. doi: 10.1084/jem.20050715

53. Barsoum IB, Koti M, Siemens DR, Graham CH. Mechanisms of Hypoxia-Mediated Immune Escape in Cancer. *Cancer Res* (2014) 74(24):7185–90. doi: 10.1158/0008-5472.CAN-14-2598

54. Stagg J, Smyth MJ. Extracellular Adenosine Triphosphate and Adenosine in Cancer. *Oncogene* (2010) 29(39):5346–58. doi: 10.1038/onc.2010.292

55. Giatromanolaki A, Kouroupi M, Pouliliou S, Mitrakas A, Hasan F, Pappa A, et al. Ectonucleotidase CD73 and CD39 Expression in Non-Small Cell Lung Cancer Relates to Hypoxia and Immunosuppressive Pathways. *Life Sci* (2020) 259:118389. doi: 10.1016/j.lfs.2020.118389

56. Cekic C, Linden J. Purinergic Regulation of the Immune System. *Nat Rev Immunol* (2016) 16(3):177–92. doi: 10.1038/nri.2016.4

57. Leone RD, Horton MR, Powell JD. Something in the Air: Hyperoxic Conditioning of the Tumor Microenvironment for Enhanced Immunotherapy. *Cancer Cell* (2015) 27(4):435–6. doi: 10.1016/j.ccell.2015.03.014

58. Sitkovsky MV. T Regulatory Cells: Hypoxia-Adenosinergic Suppression and Re-Direction of the Immune Response. *Trends Immunol* (2009) 30(3):102–8. doi: 10.1016/j.it.2008.12.002

59. Steingold JM, Hatfield SM. Targeting Hypoxia-A2A Adenosinergic Immunosuppression of Antitumor T Cells During Cancer Immunotherapy. *Front Immunol* (2020) 11:570041. doi: 10.3389/fimmu.2020.570041

60. Zhang R, Zhu F, Ren J, Huang L, Liu P, Wu G. Beclin1/PI3K-Mediated Autophagy Prevents Hypoxia-Induced Apoptosis in EAhy926 Cell Line. *Cancer Biother Radiopharm* (2011) 26(3):335–43. doi: 10.1089/cbr.2010.0814

61. Sivridis E, Koukourakis MI, Mendrinos SE, Karpouzis A, Fiska A, Kouskoukis C, et al. Beclin-1 and LC3A Expression in Cutaneous Malignant Melanomas: A Biphasic Survival Pattern for Beclin-1. *Melanoma Res* (2011) 21(3):188–95. doi: 10.1097/CMR.0b013e328346612c

62. Menrad H, Werno C, Schmid T, Copanaki E, Deller T, Dehne N, et al. Roles of Hypoxia-Inducible Factor-1alpha (HIF-1alpha) Versus HIF-2alpha in the Survival of Hepatocellular Tumor Spheroids. *Hepatology* (2010) 51(6):2183–92. doi: 10.1002/hep.23597

63. Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, et al. Regulation of Autophagy by Cytoplasmic P53. *Nat Cell Biol* (2008) 10(6):676–87. doi: 10.1038/ncb1730

64. Papandreou I, Lim AL, Laderoute K, Denko NC. Hypoxia Signals Autophagy in Tumor Cells *via* AMPK Activity, Independent of HIF-1, BNIP3, and BNIP3L. *Cell Death Differ* (2008) 15(10):1572–81. doi: 10.1038/cdd.2008.84

65. Wan XB, Fan XJ, Chen MY, Xiang J, Huang PY, Guo L, et al. Elevated Beclin 1 Expression Is Correlated With HIF-1alpha in Predicting Poor Prognosis of Nasopharyngeal Carcinoma. *Autophagy* (2010) 6(3):395–404. doi: 10.4161/auto.6.3.11303

66. Koukourakis MI, Giatromanolaki A, Sivridis E, Pitiakoudis M, Gatter KC, Harris AL. Beclin 1 Over- and Underexpression in Colorectal Cancer: Distinct Patterns Relate to Prognosis and Tumour Hypoxia. *Br J Cancer* (2010) 103(8):1209–14. doi: 10.1038/sj.bjc.6605904

67. Ma XH, Piao S, Wang D, McAfee QW, Nathanson KL, Lum JJ, et al. Measurements of Tumor Cell Autophagy Predict Invasiveness, Resistance to Chemotherapy, and Survival in Melanoma. *Clin Cancer Res* (2011) 17(10):3478–89. doi: 10.1158/1078-0432.CCR-10-2372

68. Wilson WR, Hay MP. Targeting Hypoxia in Cancer Therapy. *Nat Rev Cancer* (2011) 11(6):393–410. doi: 10.1038/nrc3064

69. Liu XW, Su Y, Zhu H, Cao J, Ding WJ, Zhao YC, et al. HIF-1α-Dependent Autophagy Protects HeLa Cells From Fenretinide (4-HPR)-Induced Apoptosis in Hypoxia. *Pharmacol Res* (2010) 62(5):416–25. doi: 10.1016/j.phrs.2010.07.002

70. Healy SJ, Gorman AM, Mousavi-Shafaei P, Gupta S, Samali A. Targeting the Endoplasmic Reticulum-Stress Response as an Anticancer Strategy. *Eur J Pharmacol* (2009) 625(1-3):234–46. doi: 10.1016/j.ejphar.2009.06.064

71. Salmon H, Remark R, Gnjatic S, Merad M. Host Tissue Determinants of Tumour Immunity. *Nat Rev Cancer* (2019) 19(4):215–27. doi: 10.1038/s41568-019-0125-9

72. Vesely MD, Kershaw MH, Schreiber RD, Smyth MJ. Natural Innate and Adaptive Immunity to Cancer. *Annu Rev Immunol* (2011) 29:235–71. doi: 10.1146/annurev-immunol-031210-101324

73. Amaravadi R, Kimmelman AC, White E. Recent Insights Into the Function of Autophagy in Cancer. *Genes Dev* (2016) 30(17):1913–30. doi: 10.1101/gad.287524.116

74. Arensman MD, Yang XS, Zhong W, Bisulco S, Upeslacis E, Rosfjord EC, et al. Anti-Tumor Immunity Influences Cancer Cell Reliance Upon ATG7. *Oncoimmunology* (2020) 9(1):1800162. doi: 10.1080/2162402X.2020.1800162

75. Michaud M, Martins I, Sukkurwala AQ, Adjemian S, Ma Y, Pellegatti P, et al. Autophagy-Dependent Anticancer Immune Responses Induced by Chemotherapeutic Agents in Mice. *Science* (2011) 334(6062):1573–7. doi: 10.1126/science.1208347

76. Le Noci V, Sommariva M, Bianchi F, Triulzi T, Tagliabue E, Balsari A, et al. Local Administration of Caloric Restriction Mimetics to Promote the Immune Control of Lung Metastases. *J Immunol Res* (2019) 2019:2015892. doi: 10.1155/2019/2015892

77. Simone BA, Palagani A, Strickland K, Ko K, Jin L, Lim MK, et al. Caloric Restriction Counteracts Chemotherapy-Induced Inflammation and Increases Response to Therapy in a Triple Negative Breast Cancer Model. *Cell Cycle* (2018) 17(13):1536–44. doi: 10.1080/15384101.2018.1471314

78. Lo Re O, Panebianco C, Porto S, Cervi C, Rappa F, Di Biase S, et al. Fasting Inhibits Hepatic Stellate Cells Activation and Potentiates Anti-Cancer Activity of Sorafenib in Hepatocellular Cancer Cells. *J Cell Physiol* (2018) 233(2):1202–12. doi: 10.1002/jcp.25987

79. Di Tano M, Raucci F, Vernieri C, Caffa I, Buono R, Fanti M, et al. Synergistic Effect of Fasting-Mimicking Diet and Vitamin C Against KRAS Mutated Cancers. *Nat Commun* (2020) 11(1):2332. doi: 10.1038/s41467-020-16243-3

80. Guerriero JL. Macrophages: Their Untold Story in T Cell Activation and Function. *Int Rev Cell Mol Biol* (2019) 342:73–93. doi: 10.1016/bs.ircmb.2018.07.001

81. Vodnala SK, Eil R, Kishton RJ, Sukumar M, Yamamoto TN, Ha NH, et al. T Cell Stemness and Dysfunction in Tumors Are Triggered by a Common Mechanism. *Science* (2019) 363(6434):eaau0135. doi: 10.1126/science.aau0135

82. Pan H, Chen L, Xu Y, Han W, Lou F, Fei W, et al. Autophagy-Associated Immune Responses and Cancer Immunotherapy. *Oncotarget* (2016) 7 (16):21235–46. doi: 10.18632/oncotarget.6908

83. Wei J, Long L, Yang K, Guy C, Shrestha S, Chen Z, et al. Autophagy Enforces Functional Integrity of Regulatory T Cells by Coupling Environmental Cues and Metabolic Homeostasis. *Nat Immunol* (2016) 17(3):277–85. doi: 10.1038/ni.3365

84. Chen P, Cescon M, Bonaldo P. Autophagy-Mediated Regulation of Macrophages and Its Applications for Cancer. *Autophagy* (2014) 10 (2):192–200. doi: 10.4161/auto.26927

85. Xu X, Araki K, Li S, Han JH, Ye L, Tan WG, et al. Autophagy Is Essential for Effector CD8(+) T Cell Survival and Memory Formation. *Nat Immunol* (2014) 15(12):1152–61. doi: 10.1038/ni.3025

86. Botbol Y, Guerrero-Ros I, Macian F. Key Roles of Autophagy in Regulating T-Cell Function. *Eur J Immunol* (2016) 46(6):1326–34. doi: 10.1002/eji.201545955

87. Murera D, Arbogast F, Arnold J, Bouis D, Muller S, Gros F. CD4 T Cell Autophagy Is Integral to Memory Maintenance. *Sci Rep* (2018) 8(1):5951. doi: 10.1038/s41598-018-23993-0

88. Rožman S, Yousefi S, Oberson K, Kaufmann T, Benarafa C, Simon HU. The Generation of Neutrophils in the Bone Marrow Is Controlled by Autophagy. *Cell Death Differ* (2015) 22(3):445–56. doi: 10.1038/cdd.2014.169

89. Krzywinska E, Kantari-Mimoun C, Kerdiles Y, Sobecki M, Isagawa T, Gotthardt D, et al. Loss of HIF-1α in Natural Killer Cells Inhibits Tumour Growth by Stimulating Non-Productive Angiogenesis. *Nat Commun* (2017) 8(1):1597. doi: 10.1038/s41467-017-01599-w

90. Emily R, Cluff JN, Collins C, Varadaraj A, Rajasekaran N. Hypoxia-Inducible Factor-1α Is Upregulated in Natural Killer Cells by Interleukin-2 and Hypoxia *via* PI3K/mTOR Signaling Pathway. *J Immunol* (2019) 202 (1):194.37.

91. Parodi M, Raggi F, Cangelosi D, Manzini C, Balsamo M, Blengio F, et al. Hypoxia Modifies the Transcriptome of Human NK Cells, Modulates Their Immunoregulatory Profile, and Influences NK Cell Subset Migration. *Front Immunol* (2018) 9:2358. doi: 10.3389/fimmu.2018.02358

92. Solocinski K, Padget MR, Fabian KP, Wolfson B, Cecchi F, Hembrough T, et al. Overcoming Hypoxia-Induced Functional Suppression of NK Cells. *J Immunother Cancer* (2020) 8(1):e000246. doi: 10.1136/jitc-2019-000246

93. Teng R, Wang Y, Lv N, Zhang D, Williamson RA, Lei L, et al. Hypoxia Impairs NK Cell Cytotoxicity Through SHP-1-Mediated Attenuation of STAT3 and ERK Signaling Pathways. *J Immunol Res* (2020) 2020:4598476. doi: 10.1155/2020/4598476

94. Lim SA, Moon Y, Shin MH, Kim TJ, Chae S, Yee C, et al. Hypoxia-Driven HIF-1α Activation Reprograms Pre-Activated NK Cells Towards Highly Potent Effector Phenotypes *via* ERK/STAT3 Pathways. *Cancers (Basel)* (2021) 13(8):1904. doi: 10.3390/cancers13081904

95. Noman MZ, Buart S, Van Pelt J, Richon C, Hasmim M, Leleu N, et al. The Cooperative Induction of Hypoxia-Inducible Factor-1 Alpha and STAT3 During Hypoxia Induced an Impairment of Tumor Susceptibility to CTL-Mediated Cell Lysis. *J Immunol* (2009) 182(6):3510–21. doi: 10.4049/jimmunol.0800841

96. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, et al. Autophagy Inhibition Enhances Therapy-Induced Apoptosis in a Myc-Induced Model of Lymphoma. *J Clin Invest* (2007) 117(2):326–36. doi: 10.1172/JCI28833

97. Noman MZ, Janji B, Kaminska B, Van Moer K, Pierson S, Przanowski P, et al. Blocking Hypoxia-Induced Autophagy in Tumors Restores Cytotoxic T-Cell Activity and Promotes Regression. *Cancer Res* (2011) 71(18):5976–86. doi: 10.1158/0008-5472.CAN-11-1094

98. Noman MZ, Janji B, Berchem G, Mami-Chouaib F, Chouaib S. Hypoxia-Induced Autophagy: A New Player in Cancer Immunotherapy? *Autophagy* (2012) 8(4):704–6. doi: 10.4161/auto.19572

99. Cui Y, Li YY, Li J, Zhang HY, Wang F, Bai X, et al. STAT3 Regulates Hypoxia-Induced Epithelial Mesenchymal Transition in Oesophageal Squamous Cell Cancer. *Oncol Rep* (2016) 36(1):108–16. doi: 10.3892/or.2016.4822

100. Viry E, Baginska J, Berchem G, Noman MZ, Medves S, Chouaib S, et al. Autophagic Degradation of GZMB/granzyme B: A New Mechanism of Hypoxic Tumor Cell Escape From Natural Killer Cell-Mediated Lysis. *Autophagy* (2014) 10(1):173–5. doi: 10.4161/auto.26924

101. Tittarelli A, Janji B, Van Moer K, Noman MZ, Chouaib S. The Selective Degradation of Synaptic Connexin 43 Protein by Hypoxia-Induced Autophagy Impairs Natural Killer Cell-Mediated Tumor Cell Killing. *J Biol Chem* (2015) 290(39):23670–9. doi: 10.1074/jbc.M115.651547

102. Gleisner MA, Navarrete M, Hofmann F, Salazar-Onfray F, Tittarelli A. Mind the Gaps in Tumor Immunity: Impact of Connexin-Mediated Intercellular Connections. *Front Immunol* (2017) 8:1067. doi: 10.3389/fimmu.2017.01067

103. Das V, Bhattacharya S, Chikkaputtaiah C, Hazra S, Pal M. The Basics of Epithelial-Mesenchymal Transition (EMT): A Study From a Structure, Dynamics, and Functional Perspective. *J Cell Physiol* (2019) 234(9):14535–55. doi: 10.1002/jcp.28160

104. Tam SY, Wu VWC, Law HKW. Hypoxia-Induced Epithelial-Mesenchymal Transition in Cancers: HIF-1α and Beyond. *Front Oncol* (2020) 10:486. doi: 10.3389/fonc.2020.00486

105. Luo M, Li JF, Yang Q, Zhang K, Wang ZW, Zheng S, et al. Stem Cell Quiescence and Its Clinical Relevance. *World J Stem Cells* (2020) 12 (11):1307–26. doi: 10.4252/wjsc.v12.i11.1307

106. Abou Khouzam R, Goutham HV, Zaarour RF, Chamseddine AN, Francis A, Buart S, et al. Integrating Tumor Hypoxic Stress in Novel and More Adaptable Strategies for Cancer Immunotherapy. *Semin Cancer Biol* (2020) 65:140–54. doi: 10.1016/j.semcancer.2020.01.003

107. Hadjimichael C, Chanoumidou K, Papadopoulou N, Arampatzi P, Papamatheakis J, Kretsovali A. Common Stemness Regulators of Embryonic and Cancer Stem Cells. *World J Stem Cells* (2015) 7(9):1150–84. doi: 10.4252/wjsc.v7.i9.1150

108. Sahlgren C, Gustafsson MV, Jin S, Poellinger L, Lendahl U. Notch Signaling Mediates Hypoxia-Induced Tumor Cell Migration and Invasion. *Proc Natl Acad Sci USA* (2008) 105(17):6392–7. doi: 10.1073/pnas.0802047105

109. Zhang Q, Bai X, Chen W, Ma T, Hu Q, Liang C, et al. Wnt/β-Catenin Signaling Enhances Hypoxia-Induced Epithelial-Mesenchymal Transition in Hepatocellular Carcinoma *via* Crosstalk With Hif-1α Signaling. *Carcinogenesis* (2013) 34(5):962–73. doi: 10.1093/carcin/bgt027

110. Bijlsma MF, Groot AP, Oduro JP, Franken RJ, Schoenmakers SHHF, Peppelenbosch MP, et al. Hypoxia Induces a Hedgehog Response Mediated by HIF-1alpha. *J Cell Mol Med* (2009) 13(8B):2053–60. doi: 10.1111/j.1582-4934.2008.00491.x

111. Shi YH, Bingle L, Gong LH, Wang YX, Corke KP, Fang WG. Basic FGF Augments Hypoxia Induced HIF-1-Alpha Expression and VEGF Release in T47D Breast Cancer Cells. *Pathology* (2007) 39(4):396–400. doi: 10.1080/00313020701444549

112. Zhao W, Li Y, Zhang X. Stemness-Related Markers in Cancer. *Cancer Transl Med* (2017) 3(3):87–95. doi: 10.4103/ctm.ctm_69_16

113. Guo Q, Yang C, Gao F. The State of CD44 Activation in Cancer Progression and Therapeutic Targeting. *FEBS J* (2021). doi: 10.1111/febs.16179

114. Natesh J, Chandola C, Meeran SM, Neerathilingam M. Targeted Delivery of Doxorubicin Through CD44 Aptamer to Cancer Cells. *Ther Delivery* (2021) 12(10):693–703. doi: 10.4155/tde-2021-0038

115. Glumac PM, LeBeau AM. The Role of CD133 in Cancer: A Concise Review. *Clin Transl Med* (2018) 7(1):18. doi: 10.1186/s40169-018-0198-1

116. Terry S, Engelsen AST, Buart S, Elsayed WS, Venkatesh GH, Chouaib S. Hypoxia-Driven Intratumor Heterogeneity and Immune Evasion. *Cancer Lett* (2020) 492:1–10. doi: 10.1016/j.canlet.2020.07.004

117. Hasmim M, Noman MZ, Messai Y, Bordereaux D, Gros G, Baud V, et al. Cutting Edge: Hypoxia-Induced Nanog Favors the Intratumoral Infiltration of Regulatory T Cells and Macrophages *via* Direct Regulation of TGF-β1. *J Immunol* (2013) 191(12):5802–6. doi: 10.4049/jimmunol.1302140

118. Terry S, Buart S, Tan TZ, Gros G, Noman MZ, Lorens JB, et al. Acquisition of Tumor Cell Phenotypic Diversity Along the EMT Spectrum Under Hypoxic Pressure: Consequences on Susceptibility to Cell-Mediated Cytotoxicity. *Oncoimmunology* (2017) 6(2):e1271858. doi: 10.1080/2162402X.2016.1271858

119. Terry S, Abdou A, Engelsen AST, Buart S, Dessen P, Corgnac S, et al. AXL Targeting Overcomes Human Lung Cancer Cell Resistance to NK- and CTL-Mediated Cytotoxicity. *Cancer Immunol Res* (2019) 7(11):1789–802. doi: 10.1158/2326-6066.CIR-18-0903

120. Axelrod H, Pienta KJ. Axl as a Mediator of Cellular Growth and Survival. *Oncotarget* (2014) 5(19):8818–52. doi: 10.18632/oncotarget.2422

121. Schoumacher M, Burbridge M. Key Roles of AXL and MER Receptor Tyrosine Kinases in Resistance to Multiple Anticancer Therapies. *Curr Oncol Rep* (2017) 19(3):19. doi: 10.1007/s11912-017-0579-4

122. Goyette MA, Elkholi IE, Apcher C, Kuasne H, Rothlin CV, Muller WJ, et al. Targeting Axl Favors an Antitumorigenic Microenvironment That Enhances Immunotherapy Responses by Decreasing Hif-1α Levels. *Proc Natl Acad Sci USA* (2021) 118(29):e2023868118. doi: 10.1073/pnas.2023868118

123. Samanta D, Park Y, Ni X, Li H, Zahnow CA, Gabrielson E, et al. Chemotherapy Induces Enrichment of CD47. *Proc Natl Acad Sci USA* (2018) 115(6):E1239–E48. doi: 10.1073/pnas.1718197115

124. Noman MZ, Van Moer K, Marani V, Gemmill RM, Tranchevent LC, Azuaje F, et al. CD47 Is a Direct Target of SNAI1 and ZEB1 and Its Blockade Activates the Phagocytosis of Breast Cancer Cells Undergoing EMT. *Oncoimmunology* (2018) 7(4):e1345415. doi: 10.1080/2162402X.2017.1345415

125. Zhang D, Tang DG, Rycaj K. Cancer Stem Cells: Regulation Programs, Immunological Properties and Immunotherapy. *Semin Cancer Biol* (2018) 52(Pt 2):94–106. doi: 10.1016/j.semcancer.2018.05.001

126. Faget J, Groeneveld S, Boivin G, Sankar M, Zangger N, Garcia M, et al. Neutrophils and Snail Orchestrate the Establishment of a Pro-Tumor Microenvironment in Lung Cancer. *Cell Rep* (2017) 21(11):3190–204. doi: 10.1016/j.celrep.2017.11.052

127. Silva-Filho AF, Sena WLB, Lima LRA, Carvalho LVN, Pereira MC, Santos LGS, et al. Glycobiology Modifications in Intratumoral Hypoxia: The Breathless Side of Glycans Interaction. *Cell Physiol Biochem* (2017) 41(5):1801–29. doi: 10.1159/000471912

128. Arriagada C, Silva P, Torres VA. Role of Glycosylation in Hypoxia-Driven Cell Migration and Invasion. *Cell Adh Migr* (2019) 13(1):13–22. doi: 10.1080/19336918.2018.1491234

129. Pereira MS, Alves I, Vicente M, Campar A, Silva MC, Padrao NA, et al. Glycans as Key Checkpoints of T Cell Activity and Function. *Front Immunol* (2018) 9:2754. doi: 10.3389/fimmu.2018.02754

130. Madsen CB, Petersen C, Lavrsen K, Harndahl M, Buus S, Clausen H, et al. Cancer Associated Aberrant Protein O-Glycosylation Can Modify Antigen Processing and Immune Response. *PloS One* (2012) 7(11):e50139. doi: 10.1371/journal.pone.0050139

131. Silva MC, Fernandes A, Oliveira M, Resende C, Correia A, de-Freitas-Junior JC, et al. Glycans as Immune Checkpoints: Removal of Branched N-Glycans Enhances Immune Recognition Preventing Cancer Progression. *Cancer Immunol Res* (2020) 8(11):1407–25. doi: 10.1158/2326-6066.CIR-20-0264

132. Huang Y, Zhang HL, Li ZL, Du T, Chen YH, Wang Y, et al. FUT8-Mediated Aberrant N-Glycosylation of B7H3 Suppresses the Immune Response in Triple-Negative Breast Cancer. *Nat Commun* (2021) 12(1):2672. doi: 10.1038/s41467-021-22618-x

133. Peixoto A, Fernandes E, Gaiteiro C, Lima L, Azevedo R, Soares J, et al. Hypoxia Enhances the Malignant Nature of Bladder Cancer Cells and Concomitantly Antagonizes Protein O-Glycosylation Extension. *Oncotarget* (2016) 7(39):63138–57. doi: 10.18632/oncotarget.11257

134. Peixoto A, Ferreira D, Azevedo R, Freitas R, Fernandes E, Relvas-Santos M, et al. Glycoproteomics Identifies HOMER3 as a Potentially Targetable Biomarker Triggered by Hypoxia and Glucose Deprivation in Bladder Cancer. *J Exp Clin Cancer Res* (2021) 40(1):191. doi: 10.1186/s13046-021-01988-6

135. Labrada M, Dorvignit D, Hevia G, Rodriguez-Zhurbenko N, Hernandez AM, Vazquez AM, et al. GM3(Neu5Gc) Ganglioside: An Evolution Fixed Neoantigen for Cancer Immunotherapy. *Semin Oncol* (2018) 45(1-2):41–51. doi: 10.1053/j.seminoncol.2018.04.003

136. Dorvignit D, Boligan KF, Relova-Hernandez E, Clavell M, Lopez A, Labrada M, et al. Antitumor Effects of the GM3(Neu5Gc) Ganglioside-Specific Humanized Antibody 14f7ht Against Cmah-Transfected Cancer Cells. *Sci Rep* (2019) 9(1):9921. doi: 10.1038/s41598-019-46148-1

137. Yin J, Hashimoto A, Izawa M, Miyazaki K, Chen GY, Takematsu H, et al. Hypoxic Culture Induces Expression of Sialin, a Sialic Acid Transporter, and Cancer-Associated Gangliosides Containing Non-Human Sialic Acid on Human Cancer Cells. *Cancer Res* (2006) 66(6):2937–45. doi: 10.1158/0008-5472.CAN-05-2615

138. Bousquet PA, Sandvik JA, Jeppesen Edin NF, Krengel U. Hypothesis: Hypoxia Induces *De Novo* Synthesis of NeuGc Gangliosides in Humans Through CMAH Domain Substitute. *Biochem Biophys Res Commun* (2018) 495(1):1562–6. doi: 10.1016/j.bbrc.2017.11.183

139. Groux-Degroote S, Delannoy P. Cancer-Associated Glycosphingolipids as Tumor Markers and Targets for Cancer Immunotherapy. *Int J Mol Sci* (2021) 22(11):6145. doi: 10.3390/ijms22116145

140. Perfahl H, Byrne HM, Chen T, Estrella V, Alarcon T, Lapin A, et al. Multiscale Modelling of Vascular Tumour Growth in 3D: The Roles of Domain Size and Boundary Conditions. *PloS One* (2011) 6(4):e14790. doi: 10.1371/journal.pone.0014790

141. Ribatti D, Nico B, Crivellato E, Roccaro AM, Vacca A. The History of the Angiogenic Switch Concept. *Leukemia* (2007) 21(1):44–52. doi: 10.1038/sj.leu.2404402

142. Nikitenko LL. Vascular Endothelium in Cancer. *Cell Tissue Res* (2009) 335(1):223–40. doi: 10.1007/s00441-008-0707-4

143. Krock BL, Skuli N, Simon MC. Hypoxia-Induced Angiogenesis: Good and Evil. *Genes Cancer* (2011) 2(12):1117–33. doi: 10.1177/1947601911423654

144. Zuazo-Gaztelu I, Casanovas O. Unraveling the Role of Angiogenesis in Cancer Ecosystems. *Front Oncol* (2018) 8:248. doi: 10.3389/fonc.2018.00248

145. Carmona-Rodriguez L, Martinez-Rey D, Mira E, Manes S. SOD3 Boosts T Cell Infiltration by Normalizing the Tumor Endothelium and Inducing Laminin-Alpha4. *Oncoimmunology* (2020) 9(1):1794163. doi: 10.1080/2162402X.2020.1794163

146. Griffioen AW, Damen CA, Blijham GH, Groenewegen G. Tumor Angiogenesis Is Accompanied by a Decreased Inflammatory Response of Tumor-Associated Endothelium. *Blood* (1996) 88(2):667–73. doi: 10.1182/blood.V88.2.667.bloodjournal882667

147. Peeters CF, Ruers TJ, Westphal JR, de Waal RM. Progressive Loss of Endothelial P-Selectin Expression With Increasing Malignancy in Colorectal Cancer. *Lab Invest* (2005) 85(2):248–56. doi: 10.1038/labinvest.3700217

148. Wu X, Giobbie-Hurder A, Liao X, Lawrence D, McDermott D, Zhou J, et al. VEGF Neutralization Plus CTLA-4 Blockade Alters Soluble and Cellular Factors Associated With Enhancing Lymphocyte Infiltration and Humoral Recognition in Melanoma. *Cancer Immunol Res* (2016) 4(10):858–68. doi: 10.1158/2326-6066.CIR-16-0084

149. Morita Y, Zhang R, Leslie M, Adhikari S, Hasan N, Chervoneva I, et al. Pathologic Evaluation of Tumor-Associated Macrophage Density and Vessel Inflammation in Invasive Breast Carcinomas. *Oncol Lett* (2017) 14(2):2111–8. doi: 10.3892/ol.2017.6466

150. Nummer D, Suri-Payer E, Schmitz-Winnenthal H, Bonertz A, Galindo L, Antolovich D, et al. Role of Tumor Endothelium in CD4+ CD25+ Regulatory T Cell Infiltration of Human Pancreatic Carcinoma. *J Natl Cancer Inst* (2007) 99(15):1188–99. doi: 10.1093/jnci/djm064

151. Morita Y, Leslie M, Kameyama H, Lokesh GLR, Ichimura N, Davis R, et al. Functional Blockade of E-Selectin in Tumor-Associated Vessels Enhances Anti-Tumor Effect of Doxorubicin in Breast Cancer. *Cancers (Basel)* (2020) 12(3):725. doi: 10.3390/cancers12030725

152. Lambrechts D, Wauters E, Boeckx B, Aibar S, Nittner D, Burton O, et al. Phenotype Molding of Stromal Cells in the Lung Tumor Microenvironment. *Nat Med* (2018) 24(8):1277–89. doi: 10.1038/s41591-018-0096-5

153. Platel V, Faure S, Corre I, Clere N. Endothelial-To-Mesenchymal Transition (EndoMT): Roles in Tumorigenesis, Metastatic Extravasation and Therapy Resistance. *J Oncol* (2019) 2019:8361945. doi: 10.1155/2019/8361945

154. Liu S, Qin T, Liu Z, Wang J, Jia Y, Feng Y, et al. Anlotinib Alters Tumor Immune Microenvironment by Downregulating PD-L1 Expression on Vascular Endothelial Cells. *Cell Death Dis* (2020) 11(5):309. doi: 10.1038/s41419-020-2511-3

155. Hirsch L, Flippot R, Escudier B, Albiges L. Immunomodulatory Roles of VEGF Pathway Inhibitors in Renal Cell Carcinoma. *Drugs* (2020) 80 (12):1169–81. doi: 10.1007/s40265-020-01327-7

156. Suzuki H, Onishi H, Wada J, Yamasaki A, Tanaka H, Nakano K, et al. VEGFR2 Is Selectively Expressed by FOXP3high CD4+ Treg. *Eur J Immunol* (2010) 40(1):197–203. doi: 10.1002/eji.200939887

157. Voron T, Colussi O, Marcheteau E, Pernot S, Nizard M, Pointet AL, et al. VEGF-A Modulates Expression of Inhibitory Checkpoints on CD8+ T Cells in Tumors. *J Exp Med* (2015) 212(2):139–48. doi: 10.1084/jem.20140559

158. Murakami M, Simons M. Fibroblast Growth Factor Regulation of Neovascularization. *Curr Opin Hematol* (2008) 15(3):215–20. doi: 10.1097/MOH.0b013e3282f97d98

159. Im JH, Buzzelli JN, Jones K, Franchini F, Gordon-Weeks A, Markelc B, et al. FGF2 Alters Macrophage Polarization, Tumour Immunity and Growth and can be Targeted During Radiotherapy. *Nat Commun* (2020) 11(1):4064. doi: 10.1038/s41467-020-17914-x

160. Auletta JJ, Zale EA, Welter JF, Solchaga LA. Fibroblast Growth Factor-2 Enhances Expansion of Human Bone Marrow-Derived Mesenchymal Stromal Cells Without Diminishing Their Immunosuppressive Potential. *Stem Cells Int* (2011) 2011:235176. doi: 10.4061/2011/235176

161. Palakurthi S, Kuraguchi M, Zacharek SJ, Zudaire E, Huang W, Bonal DM, et al. The Combined Effect of FGFR Inhibition and PD-1 Blockade Promotes Tumor-Intrinsic Induction of Antitumor Immunity. *Cancer Immunol Res* (2019) 7(9):1457–71. doi: 10.1158/2326-6066.CIR-18-0595

162. Raica M, Cimpean AM. Platelet-Derived Growth Factor (PDGF)/PDGF Receptors (PDGFR) Axis as Target for Antitumor and Antiangiogenic Therapy. *Pharm (Basel)* (2010) 3(3):572–99. doi: 10.3390/ph3030572

163. Heldin CH. Targeting the PDGF Signaling Pathway in Tumor Treatment. *Cell Commun Signal* (2013) 11:97. doi: 10.1186/1478-811X-11-97

164. Agrawal S, Ganguly S, Hajian P, Cao JN, Agrawal A. PDGF Upregulates CLEC-2 to Induce T Regulatory Cells. *Oncotarget* (2015) 6(30):28621–32. doi: 10.18632/oncotarget.5765

165. Shi J, Wei PK. Interleukin-8: A Potent Promoter of Angiogenesis in Gastric Cancer. *Oncol Lett* (2016) 11(2):1043–50. doi: 10.3892/ol.2015.4035

166. David JM, Dominguez C, Hamilton DH, Palena C. The IL-8/IL-8r Axis: A Double Agent in Tumor Immune Resistance. *Vaccines (Basel)* (2016) 4(3):22. doi: 10.3390/vaccines4030022

167. Luoto KR, Kumareswaran R, Bristow RG. Tumor Hypoxia as a Driving Force in Genetic Instability. *Genome Integr* (2013) 4(1):5. doi: 10.1186/2041-9414-4-5

168. Scanlon SE, Glazer PM. Multifaceted Control of DNA Repair Pathways by the Hypoxic Tumor Microenvironment. *DNA Repair (Amst)* (2015) 32:180–9. doi: 10.1016/j.dnarep.2015.04.030

169. Francis A, Venkatesh GH, Zaarour RF, Zeinelabdin NA, Nawafleh HH, Prasad P, et al. Tumor Hypoxia: A Key Determinant of Microenvironment Hostility and a Major Checkpoint During the Antitumor Response. *Crit Rev Immunol* (2018) 38(6):505–24. doi: 10.1615/CritRevImmunol.2019 030168

170. Ng N, Purshouse K, Foskolou IP, Olcina MM, Hammond EM. Challenges to DNA Replication in Hypoxic Conditions. *FEBS J* (2018) 285(9):1563–71. doi: 10.1111/febs.14377

171. Bhandari V, Li CH, Bristow RG, Boutros PC, Consortium P. Divergent Mutational Processes Distinguish Hypoxic and Normoxic Tumours. *Nat Commun* (2020) 11(1):737. doi: 10.1038/s41467-019-14052-x

172. Gerlinger M, Rowan AJ, Horswell S, Math M, Larkin J, Endesfelder D, et al. Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing. *N Engl J Med* (2012) 366(10):883–92. doi: 10.1056/NEJMoa1113205

173. Dentro SC, Leshchiner I, Haase K, Tarabichi M, Wintersinger J, Deshwar AG, et al. Characterizing Genetic Intra-Tumor Heterogeneity Across 2,658 Human Cancer Genomes. *Cell* (2021) 184(8):2239–54.e39. doi: 10.1016/j.cell.2021.03.009

174. Zhang J, Wu T, Simon J, Takada M, Saito R, Fan C, et al. VHL Substrate Transcription Factor ZHX2 as an Oncogenic Driver in Clear Cell Renal Cell Carcinoma. *Science* (2018) 361(6399):290–5. doi: 10.1126/science.aap8411

175. Bratslavsky G, Sudarshan S, Neckers L, Linehan WM. Pseudohypoxic Pathways in Renal Cell Carcinoma. *Clin Cancer Res* (2007) 13(16):4667–71. doi: 10.1158/1078-0432.CCR-06-2510

176. Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, et al. Genomic Architecture and Evolution of Clear Cell Renal Cell Carcinomas Defined by Multiregion Sequencing. *Nat Genet* (2014) 46(3):225–33. doi: 10.1038/ng.2891

177. Sansregret L, Vanhaesebroeck B, Swanton C. Determinants and Clinical Implications of Chromosomal Instability in Cancer. *Nat Rev Clin Oncol* (2018) 15(3):139–50. doi: 10.1038/nrclinonc.2017.198

178. Madan E, Peixoto ML, Dimitrion P, Eubank TD, Yekelchyk M, Talukdar S, et al. Cell Competition Boosts Clonal Evolution and Hypoxic Selection in Cancer. *Trends Cell Biol* (2020) 30(12):967–78. doi: 10.1016/j.tcb.2020.10.002

179. Graeber TG, Osmanian C, Jacks T, Housman DE, Koch CJ, Lowe SW, et al. Hypoxia-Mediated Selection of Cells With Diminished Apoptotic Potential in Solid Tumours. *Nature* (1996) 379(6560):88–91. doi: 10.1038/379088a0

180. Vito A, El-Sayes N, Mossman K. Hypoxia-Driven Immune Escape in the Tumor Microenvironment. *Cells* (2020) 9(4):992. doi: 10.3390/cells9040992

181. Rofstad EK, Johnsen NM, Lyng H. Hypoxia-Induced Tetraploidisation of a Diploid Human Melanoma Cell Line In Vitro. *Br J Cancer Suppl* (1996) 27:S136–9.

182. Caracciolo D, Riillo C, Arbitrio M, Di Martino MT, Tagliaferri P, Tassone P. Error-Prone DNA Repair Pathways as Determinants of Immunotherapy Activity: An Emerging Scenario for Cancer Treatment. *Int J Cancer* (2020) 147(10):2658–68. doi: 10.1002/ijc.33038

183. Kakoti S, Sato H, Laskar S, Yasuhara T, Shibata A. DNA Repair and Signaling in Immune-Related Cancer Therapy. *Front Mol Biosci* (2020) 7:205. doi: 10.3389/fmolb.2020.00205

184. Bader SB, Ma TS, Simpson CJ, Liang J, Maezono SEB, Olcina MM, et al. Replication Catastrophe Induced by Cyclic Hypoxia Leads to Increased APOBEC3B Activity. *Nucleic Acids Res* (2021) 49(13):7492–506. doi: 10.1093/nar/gkab551

185. Wang P, Chen Y, Wang C. Beyond Tumor Mutation Burden: Tumor Neoantigen Burden as a Biomarker for Immunotherapy and Other Types of Therapy. *Front Oncol* (2021) 11:672677. doi: 10.3389/fonc.2021.672677

186. Hassan Venkatesh G, Bravo P, Shaaban Moustafa Elsayed W, Amirtharaj F, Wojtas B, Abou Khouzam R, et al. Hypoxia Increases Mutational Load of Breast Cancer Cells Through Frameshift Mutations. *Oncoimmunology* (2020) 9(1):1750750. doi: 10.1080/2162402X.2020.1750750

187. Hassan Venkatesh G, Abou Khouzam R, Shaaban Moustafa Elsayed W, Ahmed Zeinelabdin N, Terry S, Chouaib S. Tumor Hypoxia: An Important Regulator of Tumor Progression or a Potential Modulator of Tumor Immunogenicity? *Oncoimmunology* (2021) 10(1):1974233. doi: 10.1080/2162402X.2021.1974233

188. Liu CC, Steen CB, Newman AM. Computational Approaches for Characterizing the Tumor Immune Microenvironment. *Immunology* (2019) 158(2):70–84. doi: 10.1111/imm.13101

189. Zhang F, Wang X, Bai Y, Hu H, Yang Y, Wang J, et al. Development and Validation of a Hypoxia-Related Signature for Predicting Survival Outcomes in Patients With Bladder Cancer. *Front Genet* (2021) 12:670384. doi: 10.3389/fgene.2021.670384

190. Liu Z, Tang Q, Qi T, Othmane B, Yang Z, Chen J, et al. A Robust Hypoxia Risk Score Predicts the Clinical Outcomes and Tumor Microenvironment Immune Characters in Bladder Cancer. *Front Immunol* (2021) 12:725223. doi: 10.3389/fimmu.2021.725223

191. Qi L, Chen J, Yang Y, Hu W. Hypoxia Correlates With Poor Survival and M2 Macrophage Infiltration in Colorectal Cancer. *Front Oncol* (2020) 10:566430. doi: 10.3389/fonc.2020.566430

192. Pei JP, Zhang CD, Yusupu M, Zhang C, Dai DQ. Screening and Validation of the Hypoxia-Related Signature of Evaluating Tumor Immune Microenvironment and Predicting Prognosis in Gastric Cancer. *Front Immunol* (2021) 12:705511. doi: 10.3389/fimmu.2021.705511

193. Lin W, Wu S, Chen X, Ye Y, Weng Y, Pan Y, et al. Characterization of Hypoxia Signature to Evaluate the Tumor Immune Microenvironment and Predict Prognosis in Glioma Groups. *Front Oncol* (2020) 10:796. doi: 10.3389/fonc.2020.00796

194. Zhang Q, Qiao L, Liao J, Liu Q, Liu P, Liu L. A Novel Hypoxia Gene Signature Indicates Prognosis and Immune Microenvironments Characters

in Patients With Hepatocellular Carcinoma. *J Cell Mol Med* (2021) 25 (8):3772–84. doi: 10.1111/jcmm.16249

195. Mo Z, Yu L, Cao Z, Hu H, Luo S, Zhang S. Identification of a Hypoxia-Associated Signature for Lung Adenocarcinoma. *Front Genet* (2020) 11:647. doi: 10.3389/fgene.2020.00647

196. Shou Y, Yang L, Yang Y, Zhu X, Li F, Xu J. Determination of Hypoxia Signature to Predict Prognosis and the Tumor Immune Microenvironment in Melanoma. *Mol Omics* (2021) 17(2):307–16. doi: 10.1039/D0MO00159G

197. Abou Khouzam R, Rao SP, Venkatesh GH, Zeinelabdin NA, Buart S, Meylan M, et al. An Eight-Gene Hypoxia Signature Predicts Survival in Pancreatic Cancer and Is Associated With an Immunosuppressed Tumor Microenvironment. *Front Immunol* (2021) 12:680435. doi: 10.3389/fimmu.2021.680435

198. Finotello F, Trajanoski Z. Quantifying Tumor-Infiltrating Immune Cells From Transcriptomics Data. *Cancer Immunol Immunother* (2018) 67 (7):1031–40. doi: 10.1007/s00262-018-2150-z

199. Jiménez-Sánchez A, Cast O, Miller ML. Comprehensive Benchmarking and Integration of Tumor Microenvironment Cell Estimation Methods. *Cancer Res* (2019) 79(24):6238–46. doi: 10.1158/0008-5472.CAN-18-3560

200. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, et al. Systematic RNA Interference Reveals That Oncogenic KRAS-Driven Cancers Require TBK1. *Nature* (2009) 462(7269):108–12. doi: 10.1038/nature08460

201. Vallania F, Tam A, Lofgren S, Schaffert S, Azad TD, Bongen E, et al. Leveraging Heterogeneity Across Multiple Datasets Increases Cell-Mixture Deconvolution Accuracy and Reduces Biological and Technical Biases. *Nat Commun* (2018) 9(1):4735. doi: 10.1038/s41467-018-07242-6

202. Bolis M, Vallerga A, Fratelli M. Computational Deconvolution of Transcriptomic Data for the Study of Tumor-Infiltrating Immune Cells. *Int J Biol Markers* (2020) 35(1_suppl):20–2. doi: 10.1177/1724600820903317

203. Yoshihara K, Shahmoradgoli M, Martínez E, Vegesna R, Kim H, Torres-Garcia W, et al. Inferring Tumour Purity and Stromal and Immune Cell Admixture From Expression Data. *Nat Commun* (2013) 4:2612. doi: 10.1038/ncomms3612

204. Abbas AR, Baldwin D, Ma Y, Ouyang W, Gurney A, Martin F, et al. Immune Response *In Silico* (IRIS): Immune-Specific Genes Identified From a Compendium of Microarray Expression Data. *Genes Immun* (2005) 6 (4):319–31. doi: 10.1038/sj.gene.6364173

205. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, et al. Robust Enumeration of Cell Subsets From Tissue Expression Profiles. *Nat Methods* (2015) 12(5):453–7. doi: 10.1038/nmeth.3337

206. Newman AM, Steen CB, Liu CL, Gentles AJ, Chaudhuri AA, Scherer F, et al. Determining Cell Type Abundance and Expression From Bulk Tissues With Digital Cytometry. *Nat Biotechnol* (2019) 37(7):773–82. doi: 10.1038/s41587-019-0114-2

207. Becht E, Giraldo NA, Lacroix L, Buttard B, Elarouci N, Petitprez F, et al. Estimating the Population Abundance of Tissue-Infiltrating Immune and Stromal Cell Populations Using Gene Expression. *Genome Biol* (2016) 17 (1):218. doi: 10.1186/s13059-016-1070-5

208. Avila Cobos F, Vandesompele J, Mestdagh P, De Preter K. Computational Deconvolution of Transcriptomics Data From Mixed Cell Populations. *Bioinformatics* (2018) 34(11):1969–79. doi: 10.1093/bioinformatics/bty019

209. Miao YR, Zhang Q, Lei Q, Luo M, Xie GY, Wang H, et al. ImmuCellAI: A Unique Method for Comprehensive T-Cell Subsets Abundance Prediction and Its Application in Cancer Immunotherapy. *Adv Sci (Weinh)* (2020) 7 (7):1902880. doi: 10.1002/advs.201902880

210. Jiang P, Gu S, Pan D, Fu J, Sahu A, Hu X, et al. Signatures of T Cell Dysfunction and Exclusion Predict Cancer Immunotherapy Response. *Nat Med* (2018) 24(10):1550–8. doi: 10.1038/s41591-018-0136-1

211. Chen X, Yan L, Lu Y, Jiang F, Zeng N, Yang S, et al. A Hypoxia Signature for Predicting Prognosis and Tumor Immune Microenvironment in Adrenocortical Carcinoma. *J Oncol* (2021) 2021:2298973. doi: 10.1155/2021/2298973

212. Jiang M, Ren L, Chen Y, Wang H, Wu H, Cheng S, et al. Identification of a Hypoxia-Related Signature for Predicting Prognosis and the Immune Microenvironment in Bladder Cancer. *Front Mol Biosci* (2021) 8:613359. doi: 10.3389/fmolb.2021.613359

213. Sun X, Zhou Z, Zhang Y, Wang J, Zhao X, Jin L, et al. Identification and Validation of a Hypoxia-Related Prognostic and Immune Microenvironment Signature in Bladder Cancer. *Cancer Cell Int* (2021) 21(1):251. doi: 10.1186/s12935-021-01954-4

214. Gong PJ, Shao YC, Huang SR, Zeng YF, Yuan XN, Xu JJ, et al. Corrigendum: Hypoxia-Associated Prognostic Markers and Competing Endogenous RNA Co-Expression Networks in Breast Cancer. *Front Oncol* (2020) 10:637481. doi: 10.3389/fonc.2020.579868

215. Zhang Y, Yang F, Peng X, Li X, Luo N, Zhu W, et al. Hypoxia Constructing the Prognostic Model of Colorectal Adenocarcinoma and Related to the Immune Microenvironment. *Front Cell Dev Biol* (2021) 9:665364. doi: 10.3389/fcell.2021.665364

216. Chen YF, Yu ZL, Lv MY, Zheng B, Tan YX, Ke J, et al. Genome-Wide Analysis Reveals Hypoxic Microenvironment Is Associated With Immunosuppression in Poor Survival of Stage II/III Colorectal Cancer Patients. *Front Med (Lausanne)* (2021) 8:686885. doi: 10.3389/fmed.2021.686885

217. Zhang L, Wang S, Wang Y, Zhao W, Zhang Y, Zhang N, et al. Effects of Hypoxia in Intestinal Tumors on Immune Cell Behavior in the Tumor Microenvironment. *Front Immunol* (2021) 12:645320. doi: 10.3389/fimmu.2021.645320

218. Ding Z, Li H, Yu D. Development and Validation of a Hypoxia-Related Gene Pair Signature to Predict Overall Survival in Head and Neck Squamous Cell Carcinoma. *Eur Arch Otorhinolaryngol* (2021) 278(10):3973–83. doi: 10.1007/s00405-020-06580-w

219. Deng F, Chen D, Wei X, Lu S, Luo X, He J, et al. Development and Validation of a Prognostic Classifier Based on HIF-1 Signaling for Hepatocellular Carcinoma. *Aging (Albany NY)* (2020) 12(4):3431–50. doi: 10.18632/aging.102820

220. Liu Z, Liu L, Lu T, Wang L, Li Z, Jiao D, et al. Hypoxia Molecular Characterization in Hepatocellular Carcinoma Identifies One Risk Signature and Two Nomograms for Clinical Management. *J Oncol* (2021) 2021:6664386. doi: 10.1155/2021/6664386

221. Zhang B, Tang B, Gao J, Li J, Kong L, Qin L. A Hypoxia-Related Signature for Clinically Predicting Diagnosis, Prognosis and Immune Microenvironment of Hepatocellular Carcinoma Patients. *J Transl Med* (2020) 18(1):342. doi: 10.1186/s12967-020-02492-9

222. Jiang HY, Ning G, Wang YS, Lv WB. Ahypoxia-Related Signature Enhances the Prediction of the Prognosis in Hepatocellular Carcinoma Patients and Correlates With Sorafenib Treatment Response. *Am J Transl Res* (2020) 12 (12):7762–81.

223. Zeng F, Zhang Y, Han X, Zeng M, Gao Y, Weng J. Employing Hypoxia Characterization to Predict Tumour Immune Microenvironment, Treatment Sensitivity and Prognosis in Hepatocellular Carcinoma. *Comput Struct Biotechnol J* (2021) 19:2775–89. doi: 10.1016/j.csbj.2021.03.033

224. Xu Z, Wei J, Qin F, Sun Y, Xiang W, Yuan L, et al. Hypoxia-Associated Alternative Splicing Signature in Lung Adenocarcinoma. *Epigenomics* (2021) 13(1):47–63. doi: 10.2217/epi-2020-0399

225. Huang Z, Wang S, Zhang HJ, Zhou YL, Tang X, Shi JH. Characteristics of Hypoxic Tumor Microenvironment in Non-Small Cell Lung Cancer, Involving Molecular Patterns and Prognostic Signature. *Transl Lung Cancer Res* (2021) 10(5):2132–47. doi: 10.21037/tlcr-20-1314

226. Shao J, Zhang B, Kuai L, Li Q. Integrated Analysis of Hypoxia-Associated lncRNA Signature to Predict Prognosis and Immune Microenvironment of Lung Adenocarcinoma Patients. *Bioengineered* (2021) 12(1):6186–200. doi: 10.1080/21655979.2021.1973874

227. Zhao C, Zhou Y, Ma H, Wang J, Guo H, Liu H. A Four-Hypoxia-Genes-Based Prognostic Signature for Oral Squamous Cell Carcinoma. *BMC Oral Health* (2021) 21(1):232. doi: 10.1186/s12903-021-01587-z

228. Fu Y, Bao Q, Liu Z, He G, Wen J, Liu Q, et al. Development and Validation of a Hypoxia-Associated Prognostic Signature Related to Osteosarcoma Metastasis and Immune Infiltration. *Front Cell Dev Biol* (2021) 9:633607. doi: 10.3389/fcell.2021.633607

229. Jiang F, Miao XL, Zhang XT, Yan F, Mao Y, Wu CY, et al. A Hypoxia Gene-Based Signature to Predict the Survival and Affect the Tumor Immune Microenvironment of Osteosarcoma in Children. *J Immunol Res* (2021) 2021:5523832. doi: 10.1155/2021/5523832

230. Chen X, Lan H, He D, Xu R, Zhang Y, Cheng Y, et al. Multi-Omics Profiling Identifies Risk Hypoxia-Related Signatures for Ovarian Cancer Prognosis. *Front Immunol* (2021) 12:645839. doi: 10.3389/fimmu.2021.645839

231. Ding J, He X, Cheng X, Cao G, Chen B, Chen S, et al. A 4-Gene-Based Hypoxia Signature Is Associated With Tumor Immune Microenvironment and Predicts the Prognosis of Pancreatic Cancer Patients. *World J Surg Oncol* (2021) 19(1):123. doi: 10.1186/s12957-021-02204-7

232. Zhang H, Qin C, Liu HW, Guo X, Gan H. An Effective Hypoxia-Related Long Non-Coding RNAs Assessment Model for Prognosis of Clear Cell Renal Carcinoma. *Front Oncol* (2021) 11:616722. doi: 10.3389/fonc.2021.616722

233. Chen H, Pan Y, Jin X, Chen G. Identification of a Four Hypoxia-Associated Long Non-Coding RNA Signature and Establishment of a Nomogram Predicting Prognosis of Clear Cell Renal Cell Carcinoma. *Front Oncol* (2021) 11:713346. doi: 10.3389/fonc.2021.713346

234. Zhang Z, Li Q, Wang F, Ma B, Meng Y, Zhang Q. Identifying Hypoxia Characteristics to Stratify Prognosis and Assess the Tumor Immune Microenvironment in Renal Cell Carcinoma. *Front Genet* (2021) 12:606816. doi: 10.3389/fgene.2021.606816

235. Stickels RR, Murray E, Kumar P, Li J, Marshall JL, Di Bella DJ, et al. Highly Sensitive Spatial Transcriptomics at Near-Cellular Resolution With Slide-Seqv2. *Nat Biotechnol* (2021) 39(3):313–9. doi: 10.1038/s41587-020-0739-1

236. Phillips D, Schürch CM, Khodadoust MS, Kim YH, Nolan GP, Jiang S. Highly Multiplexed Phenotyping of Immunoregulatory Proteins in the Tumor Microenvironment by CODEX Tissue Imaging. *Front Immunol* (2021) 12:687673. doi: 10.3389/fimmu.2021.687673

237. Baginska J, Viry E, Berchem G, Poli A, Noman MZ, van Moer K, et al. Granzyme B Degradation by Autophagy Decreases Tumor Cell Susceptibility to Natural Killer-Mediated Lysis Under Hypoxia. *Proc Natl Acad Sci USA* (2013) 110(43):17450–5. doi: 10.1073/pnas.1304790110

238. Noman MZ, Buart S, Romero P, Ketari S, Janji B, Mari B, et al. Hypoxia-Inducible miR-210 Regulates the Susceptibility of Tumor Cells to Lysis by Cytotoxic T Cells. *Cancer Res* (2012) 72(18):4629–41. doi: 10.1158/0008-5472.CAN-12-1383

239. Semenza GL. Hypoxia-Inducible Factors: Mediators of Cancer Progression and Targets for Cancer Therapy. *Trends Pharmacol Sci* (2012) 33(4):207–14. doi: 10.1016/j.tips.2012.01.005

240. Scholz CC, Taylor CT. Targeting the HIF Pathway in Inflammation and Immunity. *Curr Opin Pharmacol* (2013) 13(4):646–53. doi: 10.1016/j.coph.2013.04.009