



Selective suppression of inducible PFKFB3 isoforms as a strategy to inhibit tumor angiogenesis while preserving endothelial viability

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Abstract

Tumor angiogenesis is sustained by metabolic reprogramming within endothelial cells, enabling vessel sprouting in the hypoxic tumor microenvironment. The glycolytic regulator 6-phosphofructo-2-kinase/ fructose-2,6-biphosphatase 3 (PFKFB3) is a central driver of this pathological angiogenic response. While complete inhibition of PFKFB3 suppresses angiogenesis, it also disrupts endothelial viability, limiting therapeutic utility. In contrast, partial inhibition consistently reduces abnormal vessel sprouting while preserving baseline endothelial function, revealing a therapeutic window that remains mechanistically unexplained. Here, we advance a unifying framework in which tumor angiogenesis depends on inducible, context-specific PFKFB3 isoforms that are distinct from those supporting endothelial homeostasis. We synthesize evidence that tumor-derived metabolic signals, including lactate and vascular endothelial growth factor (VEGF), activate endothelial glycolysis through PFKFB3, and argue that this activation occurs within a hypoxia-driven, RNA-regulated program that likely reshapes PFKFB3 isoform expression. Isoforms with distinct regulatory domains, stability, and subcellular localization have been identified in other systems, providing a mechanistic basis for functional specialization. We propose that the apparent selectivity of partial PFKFB3 inhibition arises from preferential suppression of inducible, angiogenesis-associated isoforms rather than uniform attenuation of glycolytic flux. This interpretation resolves the long-standing paradox between anti-angiogenic efficacy and endothelial toxicity and challenges the prevailing dose-centric model of PFKFB3 targeting. Although direct isoform-specific evidence in endothelial cells remains limited, the convergence of metabolic, transcriptional, and splicing regulation strongly supports this model. Reframing PFKFB3 as an isoform-regulated metabolic program shifts the therapeutic paradigm from global enzymatic inhibition to isoform-selective modulation. This strategy offers a path toward more precise, mechanism-based anti-angiogenic therapies that suppress tumor vascularization while preserving essential endothelial function.

Keywords

PFKFB3 isoforms, Tumor angiogenesis, Endothelial glycolysis, Metabolic reprogramming, Hypoxia-inducible factor-1 α (HIF-1 α), Vascular endothelial growth factor (VEGF), Lactate signaling, Alternative splicing, Isoform-selective inhibition, Tumor microenvironment

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1. Introduction

Cancer progression is supported by extensive metabolic reprogramming that enables rapid and sustained tumor growth (1). As tumors expand, they outpace oxygen delivery and create hypoxic regions within the tumor microenvironment (TME) (2). To adapt, tumor cells shift from oxidative phosphorylation to aerobic glycolysis, allowing continued adenosine triphosphate (ATP) production under low oxygen conditions (3). This metabolic shift also generates biosynthetic intermediates that support accelerated proliferation (4). A major consequence of this is the accumulation of lactate, which acidifies the TME and is associated with conditions that favor tumor survival over normal tissue (5). Together, these metabolic changes are consistent with a microenvironment that promotes tumor

expansion and therapy resistance.

Tumor metabolic stress drives angiogenesis as an adaptive response to oxygen and nutrient deprivation. Hypoxia-inducible factor-1 alpha (HIF-1 α) is stabilized under hypoxic conditions and therefore induces transcription of pro-angiogenic factors, including vascular endothelial growth factor (VEGF) (6,7). VEGF signaling promotes endothelial migration, proliferation, and sprouting, enabling the formation of new blood vessels that restore tumor perfusion (8). In parallel, tumor-derived lactate acts as a metabolic signal that activates HIF-1 α in endothelial cells and further amplifies VEGF expression, including under conditions with a normal oxygen flow (9). Through these coupled hypoxic and metabolic signals, tumors use angiogenesis to sustain continued growth.

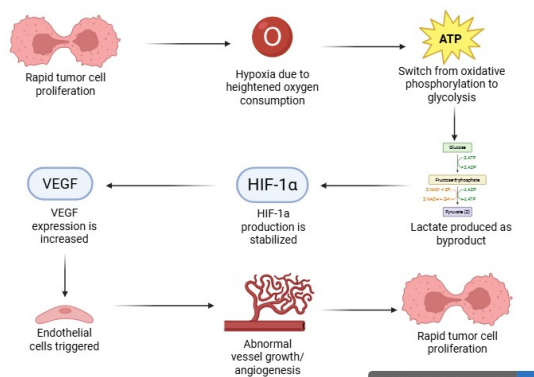


Figure 1. Tumor-driven metabolic reprogramming promotes angiogenesis. Figure created using BioRender.com.

The enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) plays a crucial role in this glycolytic shift due to its control over the availability of fructose-2,6-bisphosphate, a potent glycolysis activator that promotes increased metabolic flux in tumor cells (10). PFKFB3 expression is elevated in many cancers, enabling tumor cells to maintain

the accelerated glycolysis required for tumor proliferation and biosynthesis even under fluctuating nutrient conditions. In endothelial cells, PFKFB3 is similarly required to fuel the glycolysis-dependent migration and sprouting that drives angiogenesis (11). Tumor-derived signals such as VEGF and lactate further enhance endothelial PFKFB3 activity,

contributing to the formation of disorganized and excessive tumor vasculature (12). However, most studies quantify total PFKFB3 expression or enzymatic activity without resolving isoform-specific contributions, limiting mechanistic interpretation. As a result, PFKFB3 is often treated as a single functional entity linking tumor glycolysis to pathological angiogenesis.

Despite growing interest in PFKFB3 as a therapeutic target in tumor angiogenesis, the field has largely treated PFKFB3 as a single, uniform enzymatic entity, with therapeutic strategies focused on optimizing the degree of inhibition to balance anti-angiogenic efficacy against endothelial toxicity. However, this dose-centered framework implicitly assumes that the same molecular form of PFKFB3 supports both pathological angiogenic sprouting and baseline endothelial homeostasis, differing only in quantitative metabolic demand. This assumption remains largely untested.

Emerging evidence that PFKFB3 expression is inducible, context-dependent, and regulated at the RNA level raises the possibility that tumor-driven angiogenesis may preferentially rely on distinct, inducible PFKFB3 isoforms expressed under hypoxic and pro-angiogenic conditions, whereas endothelial viability in quiescent vessels may depend on separate, constitutively expressed isoforms. PFKFB3 is known to undergo alternative splicing and post-transcriptional regulation, producing multiple transcript variants that differ in subcellular localization, stability, and responsiveness to metabolic signaling, although their functional distinctions remain incompletely characterized (10,13,14). Multiple PFKFB3 transcript variants

have been described in non-endothelial systems, with reported differences in N-terminal regulatory sequences, subcellular localization, and protein stability. Some isoforms exhibit enhanced nuclear localization, where PFKFB3 has been linked to cell cycle progression, while others are primarily cytoplasmic and associated with glycolytic flux. These observations suggest that PFKFB3 isoforms may perform distinct metabolic and regulatory functions depending on cellular context. However, whether such isoform-specific regulation occurs in endothelial cells during tumor angiogenesis remains unresolved.

This review therefore reframes PFKFB3 as a context- and isoform-regulated metabolic program, proposing that therapeutic benefit may arise not from globally reducing PFKFB3 activity, but from selectively suppressing specific isoforms, which may offer a more precise and potentially safer strategy to inhibit pathological angiogenesis without destabilizing normal vascular function. At present, however, the specific PFKFB3 isoforms that support tumor-associated endothelial metabolism remain undefined, and most studies implicitly treat PFKFB3 as a single functional entity. Resolving isoform-specific expression and function in angiogenic versus quiescent endothelium represents a critical unmet need and a prerequisite for the rational design of isoform-selective therapeutic strategies.

2. Discussion

2.1 PFKFB3 activity is required for pathological endothelial sprouting In tumor angiogenesis Several independent studies suggest that

PFKFB3-driven glycolysis is essential for endothelial sprouting in tumor angiogenesis. When PFKFB3 is reduced in endothelial cells, glycolytic flux, filopodia formation, and migration, all decline significantly (11).

In animal models like zebrafish and mice, PFKFB3 inhibition has been associated with considerable reductions in vessel branching and sprouting (14). Schoors et al. showed that a pharmacological PFKFB3 inhibitor, 3PO, reduced the number of vascular branch points in developing zebrafish embryos. Similar results occurred in the mouse retina, which is one of the most angiogenically active tissues. Tumor-bearing models of mice also show diminished angiogenesis when PFKFB3 activity is decreased (12). A reduction in PFKFB3 activity was associated with reduced microvessel density, smaller vessel diameters, and fewer abnormal vascular loops. These findings are consistent with PFKFB3 functioning as a core metabolic driver of endothelial sprouting across diverse experimental systems.

Additionally, this metabolic dependence is consistent with the broader concept that endothelial cells rely primarily on glycolysis, rather than oxidative phosphorylation, to support angiogenic behavior even under normoxic conditions (11,16). This glycolytic bias allows endothelial cells to generate ATP rapidly and locally at sites of actin remodeling, supporting migration and sprouting.

Although these studies converge on a similar conclusion, their experimental approaches differ substantially. De Bock et al. primarily used genetic knockdown and *in vivo* endothelial

assays to demonstrate that PFKFB3 regulates endothelial tip-cell behavior, while Schoors et al. relied more heavily on pharmacological inhibition using 3PO in whole-organism angiogenesis models. Cantelmo et al., in contrast, focused on tumor-bearing mouse models and examined vascular morphology within the TME itself. These methodological differences strengthen the overall conclusion that PFKFB3 supports angiogenesis, because similar phenotypes are observed across genetic, pharmacological, and *in vivo* tumor systems. However, pharmacological inhibitors such as 3PO may also have off-target metabolic effects, which complicates interpretation of whether observed phenotypes arise exclusively from PFKFB3 inhibition (14,16).

Furthermore, most of these studies quantify total PFKFB3 expression or activity and do not distinguish between individual isoforms, limiting mechanistic interpretation. These studies largely treat PFKFB3 as a single functional entity, without resolving whether distinct PFKFB3 isoforms operate in quiescent versus tumor-associated endothelium. This distinction becomes important when considering how PFKFB3 can be therapeutically targeted without disrupting basal endothelial function.

2.2 Tumor glycolysis generates lactate, initiating endothelial angiogenic signaling

A key hallmark of cancer is the metabolic reprogramming of tumor cells toward aerobic glycolysis, often called the Warburg effect (17) (Figure 1). Instead of relying on the healthy cell's energy source, oxidative phosphorylation, many tumor cells convert glucose into lactate;

although this is a gross generalization. This process can occur even when oxygen is available in the TME. This metabolic shift is now recognized as a defining feature of cancer metabolism and is driven by oncogenic signaling pathways that promote glucose uptake and glycolytic enzyme expression (3). This shift supports rapid proliferation by generating the molecular components required for cell growth in addition to ATP (9). The resulting high lactate levels in the TME serve as signaling molecules affecting several nearby cell types, including endothelial cells, immune cells, and stromal cells (18). Beyond serving as a metabolic byproduct, the lactate functions as a signaling molecule that can directly modulate gene expression, angiogenesis, and immune suppression within the TME (5).

As tumors grow and develop hypoxic regions, they convert to glycolysis, producing more lactate (Figure 1). This lactate is exported from tumor cells and imported into endothelial cells through the monocarboxylate transporter 1 (MCT1) (9). Once inside the endothelial cells, the lactate stabilizes hypoxia-inducible factor 1- α (HIF-1 α), a subtype of HIF-1 (Figure 1). HIF-1 α , although constantly produced in healthy cells, is immediately destroyed by the cell under normal oxygen levels. However, when stabilized, HIF-1 α is no longer destroyed immediately by the cell, which increases the production of VEGF and other pro-angiogenic genes, even under normal oxygen levels (9). Sonveaux et al. showed that lactate alone, without hypoxia, increased endothelial VEGF transcription by ~3X.

Moreover, endothelial cells actively remodel their metabolism in response to these signals. During angiogenesis, they increase glycolytic activity to support migration and growth; however, because glycolysis yields less ATP per molecule of glucose than oxidative phosphorylation, cells must elevate glycolytic flux to meet the energetic demands of sprouting (9). Notably, endothelial cells generate up to 85% of their ATP through glycolysis, even in oxygen-rich conditions, highlighting the centrality of glycolytic metabolism to vascular function (11,16). Other cells within the TME, such as macrophages, further enhance angiogenesis by responding to lactate signals.

These observations suggest that tumor-derived metabolic signals generate a permissive environment for endothelial activation, setting the stage for downstream metabolic regulators such as PFKFB3 to become engaged.

2.3 Tumor metabolism activates endothelial PFKFB3 through lactate and VEGF signaling
Tumor metabolism activates endothelial PFKFB3 through a combination of lactate-mediated signaling and VEGF-driven metabolic programming. Lactate, which is released by glycolytic tumor cells, enters endothelial cells and stabilizes HIF-1 to increase the transcription of VEGF. This process is consistent with the formation of a strong pro-angiogenic TME (9). Sonveaux et al. demonstrated that blocking MCT1 prevented lactate-induced HIF-1 activation.

VEGF signaling further increases the expression of PFKFB3 in endothelial cells, pushing them into a high-flux glycolytic state that supports

sprouting and vessel formation—the earliest stages of angiogenesis (11). This regulation positions PFKFB3 as a key downstream effector of VEGF-driven angiogenesis, linking extracellular growth factor signaling to intracellular metabolic reprogramming (19). De Bock et al. also showed that VEGF increases glycolysis by increasing PFKFB3 transcription, by suppressing signaling from a signaling pathway involving the Notch receptor and Delta-like ligand 4, NOTCH-DLL4, that regulates tip and stalk specification. Tumors that generate larger amounts of lactate also have been reported to induce stronger endothelial PFKFB3 activity and produce more disorganized and unnecessary vascular networks (12). Cantelmo et al. found that tumor cells engineered to overproduce lactate generated leaky and excessively branched vasculature, correlating directly with increased endothelial PFKFB3 levels.

Together, these observations suggest that PFKFB3 is present at the intersection between tumor-derived metabolic signals and endothelial sprouting behavior (Figure 1). Importantly, this activation occurs within a tumor-specific metabolic context characterized by hypoxia, VEGF signaling, and elevated lactate. This raises the possibility that endothelial PFKFB3 expression in tumors reflects a context-dependent metabolic program distinct from the basal PFKFB3 activity that supports endothelial homeostasis in non-pathological tissues. This distinction aligns with emerging models of angiogenesis in which endothelial cell behavior is regulated by coordinated signaling and metabolic networks, rather than by growth factor signaling alone (19). Hypoxia-driven

induction of PFKFB3 is mediated in part by the transcription factor HIF-1 α , which activates glycolytic gene expression under low-oxygen conditions. Emerging evidence suggests that hypoxia and stress signaling can also influence alternative splicing through spliceosomal regulation, raising the possibility that TME signals may not only increase total PFKFB3 expression but also shift PFKFB3 isoform composition. However, current studies do not directly measure isoform-specific changes under these conditions, limiting conclusions about the role of splicing in endothelial metabolism.

2.4 Complete PFKFB3 inhibition disrupts endothelial viability and causes vascular toxicity

Evidence across multiple experimental systems suggests that full inhibition of PFKFB3 compromises endothelial cell health and disrupts vascular stability (12,14,16). Strong suppression of PFKFB3 reduces endothelial cell barrier strength and interferes with essential metabolic processes required for survival. Schoors et al. Found that high-dose 3PO decreased endothelial ATP levels by ~ 60% and triggered apoptosis markers, suggesting that PFKFB3 activity is required for basic cellular maintenance (14).

In tumor models, complete inhibition of PFKFB3 destabilizes vascular function, producing structurally inefficient and abnormal vessels that impair tissue oxygenation (12). High-dose PFKFB3 inhibitors also induce mitochondrial stress and endothelial toxicity (16). More broadly, disruption of endothelial glycolysis has been shown to impair barrier

integrity and vascular homeostasis, underscoring the reliance of endothelial cells on continuous metabolic activity for survival (15).

These findings are consistent with endothelial cells being dependent on a constitutive glycolytic program for survival, whereas pathological sprouting requires an additional, tumor-induced metabolic upregulation. Uniform inhibition of PFKFB3 therefore collapses both basal and pathological metabolic programs, thereby limiting its therapeutic utility. However, these studies do not distinguish whether the resultant toxicity arises from loss of specific PFKFB3 isoforms or from global suppression of glycolysis, leaving the underlying mechanism unresolved.

2.5 Partial PFKFB3 inhibition reduces pathological sprouting while preserving endothelial function

Partial inhibition of PFKFB3 reduces pathological angiogenesis while maintaining endothelial viability. Moderate decreases in endothelial glycolytic rates, far below the threshold of complete metabolic shutdown, are sufficient to reduce sprouting and vessel branching in both cultured endothelial cells and in *in vivo* models (14). Notably, Schoors et al. showed that a 15-20% reduction in glycolytic flux was enough to suppress sprouting, with viability remaining intact. This sensitivity suggests that angiogenic processes operate near a metabolic threshold, where small reductions in glycolytic flux can disproportionately affect sprouting behavior (14,15).

Tumor-bearing mice treated with partial PFKFB3 inhibition exhibit normalized

vasculature and improved chemotherapeutic delivery, indicating that vascular function is preserved even with the abnormal sprouting being reduced (12). Partially normalized vessels became less leaky, more aligned, and better perfused. Selective inhibitors used at submaximal doses can still suppress angiogenesis, confirming that a full glycolytic blockade is unnecessary for therapeutic benefit (16). Submaximal doses also avoid the mitochondrial toxicity seen at higher doses.

While these outcomes have been interpreted as evidence that pathological sprouting requires higher glycolytic demand than does endothelial maintenance, this interpretation assumes that PFKFB3 functions as a single enzyme devoid of isoform-specific variation across endothelial states. An alternative interpretation is that tumor angiogenesis may engage inducible, context-dependent PFKFB3 isoforms that differ from those supporting basal endothelial metabolism which may be preferentially sensitive to partial suppression. Partial inhibition may therefore disproportionately impair angiogenesis-associated PFKFB3 isoforms while sparing constitutive metabolic programs or enzyme isoforms required for endothelial survival.

2.6 Structural and regulatory diversity of PFKFB3 isoforms supports context-dependent function

PFKFB3 is not a single isoform enzyme, but exists as multiple transcript variants generated through alternative splicing and post-transcriptional regulation. Alternative splicing is a widespread mechanism for generating metabolic diversity, allowing cells to fine-tune enzyme function in response to environmental

and developmental cues (21). Studies in cancer and proliferative cell systems have identified PFKFB3 isoforms that differ in N-terminal regulatory regions, which influence subcellular localization, protein stability, and regulatory interactions (10). For example, several transcript variants, including PFKFB3-4, PFKFB3-5, and PFKFB3-11 have been described in human cells, with differences in N-terminal regulatory sequences that affect nuclear localization signals and protein turnover rates (10,14). PFKFB-4 has been reported to exhibit enhanced nuclear localization, whereas variants such as PFKFB3-5 show stronger cytoplasmic localization and are more closely associated with glycolytic flux regulation (10,14).

Additionally, nuclear-localized PFKFB3 has been implicated in cell cycle progression through localized production of fructose-2,6-bisphosphate, whereas cytoplasmic isoforms are more directly associated with glycolytic flux. These findings suggest that PFKFB3 can perform both metabolic and regulatory functions depending on isoform identity.

Additional studies in non-endothelial systems have reported that alternative PFKFB3 splice variants differ in regulatory motifs and responsiveness to cellular signaling, providing precedent for isoform-specific functional specialization (10,13). Similar isoform-specific functional differences have been observed in other metabolic enzymes, where alternative splicing alters enzymatic regulation, localization, or responsiveness to signaling pathways, suggesting that isoform-level control

may represent a broader mechanism of metabolic regulation (22,24).

Hypoxia-driven induction of PFKFB3 is mediated in part by the transcription factor HIF-1 α , which activates glycolytic gene expression under low-oxygen conditions. In addition to transcriptional regulation, hypoxia and cellular stress have been shown to influence alternative splicing through spliceosomal pathways, raising the possibility that TME signals may alter not only total PFKFB3 expression but also isoform composition. Hypoxia has been shown to globally reshape splicing patterns through regulation of spliceosomal components, linking environmental stress directly to isoform expression (23). However, most studies of PFKFB3 in endothelial cells measure total expression or enzymatic activity and do not resolve isoform-specific contributions. As a result, it remains unclear whether distinct PFKFB3 isoforms are differentially regulated in angiogenic versus quiescent endothelium.

These limitations highlight an important gap in the current literature. While existing studies demonstrate that PFKFB3 is required for endothelial sprouting, they do not distinguish whether this effect is driven by specific isoforms or by overall glycolytic flux. Additionally, these studies vary in experimental design, including differences in pharmacological inhibitors, genetic knockdown strategies, and model systems, which may contribute to variability in reported effects on endothelial metabolism and angiogenesis. Consequently, the hypothesis that tumor angiogenesis depends on inducible PFKFB3 isoforms remains untested. The

presence of structurally and functionally distinct PFKFB3 variants, however, provides a mechanistic basis for this possibility and supports further investigation into isoform-specific regulation.

2.7 Alternative mechanisms may also contribute to the effects of PFKFB3 inhibition

Although isoform-specific regulation offers one potential explanation for the differing outcomes observed between partial and complete inhibition of PFKFB3, other mechanisms could also contribute to these effects.

One possible explanation is that endothelial sprouting depends on glycolysis in a nonlinear manner. Cellular behaviors involved in angiogenesis—including migration, filopodia extension, and cytoskeletal rearrangement—require rapid ATP generation at precise intracellular locations. Because these processes are energetically demanding, they may operate close to a metabolic threshold in which even small decreases in glycolytic activity significantly disrupt sprouting while still permitting basic cellular survival functions (14,15). Within this framework, the observed impact of partial PFKFB3 inhibition could result from the high energetic sensitivity of sprouting endothelial cells rather than from selective suppression of particular enzyme variants.

Endothelial heterogeneity during vessel formation provides another potential explanation. During angiogenesis, specialized “tip cells” guide the advancing sprout, while “stalk cells” follow and form the vessel structure. Tip cells display greater migratory behavior and generally require higher metabolic

input than neighboring endothelial cells. Studies have shown that these leading cells exhibit elevated expression of glycolytic enzymes and increased glucose uptake relative to adjacent endothelial populations (11,20). As a result, glycolytic inhibition may disproportionately affect these metabolically active cells even without isoform-specific regulation.

The metabolic environment of tumors may also influence endothelial responses to glycolytic suppression. Tumor tissues frequently contain strong spatial gradients in oxygen availability, nutrient concentrations, and metabolic byproducts such as lactate. Areas, characterized by severe hypoxia or nutrient limitation could intensify the consequences of reduced glycolytic activity (5). These microenvironmental differences may therefore lead to uneven endothelial sensitivity to PFKFB3 inhibition throughout the tumor vasculature.

Importantly, these explanations are not mutually exclusive. Isoform-dependent regulation of PFKFB3 may function alongside metabolic threshold effects and microenvironmental influences. Clarifying the relative contributions of these mechanisms will likely require experimental strategies capable of simultaneously measuring glycolytic activity and isoform-specific expression at high spatial resolution or at the level of individual cells.

2.8 Mechanism-selective suppression of PFKFB3 as a therapeutic strategy

Taken together, these findings are consistent with a model in which tumor angiogenesis depends not only on elevated glycolytic flux, but

also on a tumor-contextualized PFKFB3 expression program induced by hypoxia and VEGF signaling (Figure 2). Current pharmacological approaches treat PFKFB3 as a single isoform enzymatic target, yet PFKFB3 expression is inducible, splice-regulated, and context-dependent. This raises the possibility that endothelial homeostasis and pathological sprouting may rely on distinct PFKFB3 isoforms rather than differing solely in quantitative glycolytic demand.

Importantly, existing studies do not directly distinguish between isoform-specific effects and global metabolic changes. For example, pharmacological inhibitors such as 3PO reduce overall PFKFB3 activity but do not discriminate between transcript variants, making it difficult to determine whether observed effects on angiogenesis arise from isoform-specific suppression or general reductions in glycolytic flux. Similarly, genetic knockdown approaches typically target total PFKFB3 expression rather than individual splice variants. These methodological limitations mean that current evidence is consistent with, but does not directly demonstrate, isoform-selective regulation. Therefore, isoform-specific mechanisms may be necessary but not sufficient, or alternatively sufficient but not necessary, to explain the observed phenotypes.

Several PFKFB3 transcript variants have been identified, arising from alternative splicing of the PFKFB3 gene. These isoforms primarily differ in their regulatory domains, which can influence protein stability, subcellular

localization, and responsiveness to cellular signaling. Some variants have been reported to show increased stability or altered regulatory control, suggesting that they may be differentially expressed under specific conditions such as hypoxia or cellular stress. Although their roles in endothelial cells remains poorly defined, the existence of multiple PFKFB3 isoforms supports the possibility that tumor-associated endothelium relies on inducible variants, while quiescent vessels depend on constitutive forms. This provides a mechanistic basis for why partial inhibition may preferentially suppress pathological angiogenesis while preserving normal vascular function.

From this perspective, the apparent success of partial inhibition may reflect preferential suppression of inducible, angiogenesis-associated PFKFB3 isoforms rather than simple dose-dependent attenuation of glycolysis. While the isoform-selective framework is consistent with existing data, it has not yet been directly demonstrated, and alternative explanations remain viable. Therapeutic strategies that selectively target tumor-induced PFKFB3 isoforms—such as isoform-directed suppression—could therefore provide greater specificity and reproducibility than general enzymatic inhibition (Figure 2). Reframing PFKFB3 as a context-regulated metabolic program rather than a single enzymatic node may enable more precise anti-angiogenic interventions that disrupt tumor-driven vascular remodeling while preserving essential endothelial metabolic function.

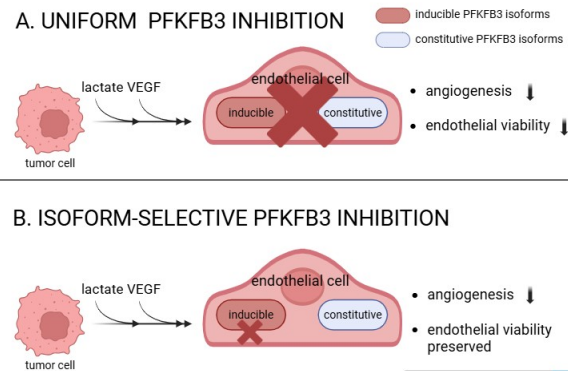


Figure 2. Uniform v.s. isoform-selective PFKFB3 inhibition.. Figure created using [BioRender.com](https://www.biorender.com).

2.9 Limitations

The isoform-selective framework proposed here has not yet been experimentally validated, and current evidence remains indirect. Most studies of PFKFB3 in endothelial cells measure total expression or enzymatic activity without resolving individual splice variants. As a result, the role of specific PFKFB3 isoforms in tumor angiogenesis versus endothelial homeostasis remains uncertain and largely speculative.

Many commonly used experimental approaches, including pharmacological inhibition (e.g. 3PO) and genetic knockdown, affect total PFKFB3 activity without distinguishing between isoforms. This makes it difficult to determine whether observed effects on angiogenesis arise primarily from isoform-specific mechanisms or from global reductions in glycolytic flux.

Additionally, alternative mechanisms, including nonlinear metabolic thresholds and endothelial heterogeneity, may also contribute to the effects observed with partial PFKFB3 inhibition. These mechanisms may be sufficient to explain some

experimental observations independent of isoform-specific regulation, or may operate in parallel with it. Distinguishing between these possibilities will require experimental approaches capable of resolving both isoform-specific expression and metabolic flux *in vivo*.

3. Conclusion

Overall, convergent evidence supports endothelial PFKFB3 as a central metabolic driver of pathological angiogenic sprouting in tumors. Tumor-derived lactate and VEGF signaling have been shown to activate high-flux glycolysis in endothelial cells, enabling rapid migration and vessel formation that sustain tumor growth. Although complete PFKFB3 inhibition suppresses angiogenesis, it also disrupts endothelial viability and vascular stability. In contrast, partial inhibition consistently reduces abnormal sprouting while preserving vascular function, suggesting that pathological angiogenesis may require a higher glycolytic demand than baseline endothelial maintenance.

These findings indicate that PFKFB3 activity in tumor endothelium may reflect a context-dependent metabolic program induced by hypoxia and pro-angiogenic signaling rather than a uniform requirement across all endothelial states. This interpretation is consistent with the ability of partial inhibition to suppress pathological angiogenesis without causing widespread vascular toxicity, supporting the therapeutic value of regulating, rather than eliminating, endothelial glycolysis in cancer.

However, current evidence does not directly distinguish whether these effects arise primarily from isoform-specific regulation or from broader metabolic constraints. Isoform-specific mechanisms may therefore be necessary but not sufficient, or alternatively sufficient but not necessary, to account for the observed phenotypes.

Future studies should directly test whether PFKFB3 isoforms differentially regulate pathological angiogenesis and endothelial homeostasis. First, transcriptomic approaches such as RNA sequencing can be used to identify PFKFB3 isoforms that are selectively expressed under hypoxic and VEGF-stimulated conditions compared to normoxic controls. Isoform-

specific expression can then be validated using RT-qPCR with junction-specific primers designed to distinguish alternatively spliced transcripts. Given the limitations of short-read RNA sequencing in resolving splice variants, long-read sequencing or targeted isoform profiling may be required for accurate characterization.

Once identified, isoform-specific knockdown strategies, including siRNA or antisense oligonucleotides targeting unique exon-exon junctions, can be used to selectively suppress individual PFKFB3 isoforms in endothelial cells. Functional outcomes, including glycolytic flux, migration, sprouting, and cell viability, can then be measured to determine whether selective suppression of inducible PFKFB3 isoforms is sufficient to inhibit angiogenesis and/or necessary to preserve endothelial survival.

These findings should be validated in tumor-bearing animal models to assess effects on vessel density, vascular normalization, and perfusion. Together, this approach would determine whether isoform-selective or context-specific PFKFB3 inhibition can be translated into safer and more effective anti-angiogenic therapies.

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