Peer-Review

Bhattacharyya, Aarshia. 2026. “Selective Suppression of Inducible PFKFB3 Isoforms as a Strategy to Inhibit Tumor Angiogenesis While Preserving Endothelial Viability.” *Journal of High School Science* 10 (1): 335–49. https://doi.org/10.64336/001c.159200

Your manuscript describes literature that already exists in the public domain. It does not hence contribute significantly to the existing corpus of knowledge in this field. It does not satisfy the Journal’s expectations of a review manuscript as described here: https://jhss.scholasticahq.com/for-authors, types of manuscripts, review papers.
You need a mechanistic insight that is not yet recognized in the public domain and inclusion of which will make the manuscript publishable as a review paper. I suggest the following: PFKFB3 expressed various protein isoforms; some inducible by HIF and some constitutively expressed. Explore the incorrect implicit assumption in the field that endothelial survival and pathological angiogenic sprouting depend on the same molecular form of PFKFB3 and differ only quantitatively in glycolytic demand.
PFKFB3 is inducible, context-dependent, and splice-regulated. Tumor endothelium operates in a fundamentally different metabolic environment than quiescent vessels. Partial inhibition may succeed ACCIDENTLY by suppressing inducible isoforms more than basal ones
Therefore the unaddressed gap is: No studies currently resolve PFKFB3 function at the isoform level during tumor angiogenesis. No therapeutic strategies exploit RNA-level specificity in endothelial metabolism
New research trajectory: Shift from dose optimization to mechanism-selective suppression. Treat angiogenesis as an RNA-regulated metabolic state, not just an enzymatic one.
Here’s a paragraph that explains this.
Isoform-selective regulation of PFKFB3 represents an unexplored therapeutic opportunity. PFKFB3 is subject to alternative splicing, and its expression is strongly inducible by hypoxia and VEGF signaling—conditions that dominate the tumor microenvironment. This raises the possibility that tumor-driven angiogenesis relies preferentially on inducible PFKFB3 splice variants, while endothelial homeostasis depends on constitutively expressed isoforms. If correct, this would imply that partial enzymatic inhibition succeeds not merely by reducing glycolytic flux, but also in parallel; suppressing inducible isoforms with higher metabolic output. Splice-directing molecules, such as antisense oligonucleotides, could therefore offer a more selective, reproducible and relatively treatment resistant strategy by reducing expression of angiogenesis-associated PFKFB3 isoforms without collapsing the basal glycolytic program required for endothelial viability.
Please also propose a testable experimental design.

Comment 1

Your manuscript describes literature that already exists in the public domain. It does not hence contribute significantly to the existing corpus of knowledge in this field. It does not satisfy the Journal’s expectations of a review manuscript as described here: https://jhss.scholasticahq.com/for-authors, types of manuscripts, review papers.

Fix

The revised manuscript has been reframed to move beyond synthesis of existing literature and instead propose a mechanistic hypothesis that is not yet explicitly addressed in the field. Specifically, it introduces the concept that endothelial PFKFB3 activity in tumor angiogenesis may be isoform-specific and regulated at the RNA level, rather than representing a uniform process. This reframing shifts the focus of the review to proposing isoform-selective metabolic control as a therapeutic strategy, addressing an unexamined gap in the literature, as the Journal’s expectations of a review manuscript state is necessary. These changes are reflected primarily in the Introduction’s final paragraph, the Discussion’s final section, and in the Conclusion–as well as being further illustrated in a newly created Figure 2.

Comment 2
You need a mechanistic insight that is not yet recognized in the public domain and inclusion of which will make the manuscript publishable as a review paper. I suggest the following: PFKFB3 expressed various protein isoforms; some inducible by HIF and some constitutively expressed. Explore the incorrect implicit assumption in the field that endothelial survival and pathological angiogenic sprouting depend on the same molecular form of PFKFB3 and differ only quantitatively in glycolytic demand.

Fix
This suggested mechanistic insight has been incorporated as a central component of the revised manuscript. It now proposes that PFKFB3 exists as multiple protein isoforms, including inducible isoforms regulated by hypoxia and HIF signaling and constitutively expressed isoforms associated with basal endothelial metabolism. The structural basis for isoform diversity is explicitly discussed, including alternative splicing and variation in regulatory domains, even though their endothelial-specific functions remain incompletely characterized. The reviewer's concern regarding the implicit assumption that endothelial survival and pathological angiogenesis rely on the same molecular form of PFKFB3 is further addressed; the revised manuscript challenges this assumption by proposing that these processes may instead depend on distinct isoforms with different regulatory properties and metabolic outputs. This concept is introduced in the Introduction and expanded in the Discussion, where prior findings on partial PFKFB3 inhibition are reinterpreted in the context of potential isoform-specific effects.

Comment 3
PFKFB3 is inducible, context-dependent, and splice-regulated. Tumor endothelium operates in a fundamentally different metabolic environment than quiescent vessels. Partial inhibition may succeed ACCIDENTLY by suppressing inducible isoforms more than basal ones.

Fix
This concept has been incorporated as a central component of the revised mechanistic framework. The revised Discussion now emphasizes that PFKFB3 is inducible, context-dependent, and subject to splice regulation, particularly under tumor-associated conditions such as hypoxia and VEGF signaling. A new mechanistic paragraph describing how HIF-1α–mediated transcriptional activation and stress-responsive spliceosomal regulation may influence both total PFKFB3 expression and isoform composition was also added. It is further proposed that tumor endothelium represents a distinct metabolic state, in which glycolytic demand and regulatory signaling differ fundamentally from quiescent vessels. Importantly, the manuscript now explicitly suggests that the observed effects of partial PFKFB3 inhibition may arise not only from reduced glycolytic flux but also from preferential suppression of inducible, angiogenesis-associated isoforms, thereby sparing constitutive isoforms required for endothelial survival.

Comment 4
Therefore the unaddressed gap is: No studies currently resolve PFKFB3 function at the isoform level during tumor angiogenesis. No therapeutic strategies exploit RNA-level specificity in endothelial metabolism.

Fix
This unaddressed gap has been explicitly incorporated into the revised manuscript. The Introduction now identifies the lack of isoform-level resolution of PFKFB3 function in tumor angiogenesis as a critical limitation in the current literature. The Discussion further highlights that existing studies treat PFKFB3 as a single enzymatic target, without considering

isoform-specific regulation. It is proposed that this gap represents a significant opportunity for advancing the field, particularly through the development of RNA-level therapeutic strategies that selectively target angiogenesis-associated isoforms. This concept is discussed in both the Discussion and Conclusion, where isoform-selective targeting is framed as a novel and unexplored direction for anti-angiogenic therapy.

Comment 5
New research trajectory: Shift from dose optimization to mechanism-selective suppression. Treat angiogenesis as an RNA-regulated metabolic state, not just an enzymatic one.

Fix
The revised manuscript incorporates this proposed research trajectory into the revised manuscript.The Discussion now reframes current anti-angiogenic strategies from dose-dependent inhibition of glycolysis toward mechanism-selective suppression of specific PFKFB3 programs. The concept that angiogenesis may represent an RNA-regulated metabolic state, in which transcriptional and post-transcriptional regulation, including alternative splicing, determine endothelial behavior in different microenvironments, is introduced. This conceptual shift is further emphasized in the Conclusion, where it is proposed that future therapeutic strategies should focus on selective modulation of PFKFB3 isoforms, rather than uniform enzymatic inhibition.

Comment 6

Here's a paragraph that explains this.

Isoform-selective regulation of PFKFB3 represents an unexplored therapeutic opportunity.

PFKFB3 is subject to alternative splicing, and its expression is strongly inducible by hypoxia and VEGF signaling—conditions that dominate the tumor microenvironment. This raises the possibility that tumor-driven angiogenesis relies preferentially on inducible PFKFB3 splice variants, while endothelial homeostasis depends on constitutively expressed isoforms. If correct, this would imply that partial enzymatic inhibition succeeds not merely by reducing glycolytic flux, but also in parallel; suppressing inducible isoforms with higher metabolic output. Splice-directing molecules, such as antisense oligonucleotides, could therefore offer a more selective, reproducible and relatively treatment resistant strategy by reducing expression of angiogenesis-associated PFKFB3 isoforms without collapsing the basal glycolytic program required for endothelial viability.
Fix
The reviewer is sincerely thanked for this detailed conceptual paragraph. These ideas have been incorporated and expanded upon throughout the revised manuscript. Specifically:
- The concept of isoform-selective regulation as a central hypothesis has been integrated
- The discussion of alternative splicing and hypoxia/VEGF-driven induction has been expanded upon
- It is proposed that tumor angiogenesis preferentially relies on inducible PFKFB3 isoforms, while endothelial homeostasis depends on the constitutive isoforms
- Partial inhibition has been reinterpreted as potentially reflecting isoform-selective effects
- Splice-directing therapeutic strategies, including antisense oligonucleotides, were introduced as a potential approach for selective targeting

These ideas are incorporated primarily in the Discussion and summarized in the Conclusion. Additionally, Figure 2 was created, which visually represents the difference in a simplified manner between uniform inhibition and isoform-selective inhibition.

Comment 7
Please also propose a testable experimental design.

Fix
A detailed, testable experimental design has been added at the end of the Conclusion, reworking the previous Future Studies paragraph. The proposed experimental framework includes identification of PFKFB3 isoforms, validation of isoform expression, functional testing of isoforms, assessment of endothelial function, and in vivo validation. This experimental design directly tests the hypothesis that distinct PFKFB3 isoforms differentially regulate angiogenesis and endothelial viability, thereby addressing the mechanistic gap identified by the reviewer.

Thank you for addressing my comments. The manuscript is much improved from its original version. It is actually almost there and when you address my additional concerns below, will make an impact in the scientific literature.
Some inconsistencies and deficiencies remain. Please address these.
1.You need alternative explanations, just so readers are aware that the isoform-selective framework may not be the only one operating. Below is a paragraph suggested by chatgpt, which I agree with; that you can insert into the manuscript under a “competing explanations” subsection. Please rewrite in your own words.
"While the isoform-selective framework provides a plausible explanation for the differential effects of partial versus complete PFKFB3 inhibition, alternative mechanisms should also be considered. One possibility is that endothelial sprouting exhibits a nonlinear dependence on glycolytic flux, such that modest reductions in glycolysis disproportionately impair energetically demanding processes like migration and filopodia formation, while basal cellular maintenance remains relatively preserved. Additionally, endothelial heterogeneity, including the distinction between tip and stalk cell phenotypes, may contribute to differential sensitivity to metabolic inhibition, independent of isoform-specific regulation. Spatial gradients in oxygen, lactate, and nutrient availability within the tumor microenvironment may further create localized metabolic thresholds that influence angiogenic behavior without requiring distinct molecular forms of PFKFB3. These alternative explanations suggest that the observed effects of partial inhibition could arise from systems-level metabolic constraints rather than isoform-specific mechanisms alone. However, these models are not mutually exclusive; isoform-selective regulation may operate in parallel with metabolic threshold effects, and distinguishing between these possibilities will require experimental approaches capable of resolving both glycolytic flux and isoform-specific expression at single-cell or spatial resolution. "
2.The isoform biology is still underdeveloped. You need to present What isoforms exist (even from non-endothelial studies)? Structural differences (N-terminus, localization)? Any functional precedent? You can do this with literature references. It does not need to be exhaustive but must suggest that the isoform hypothesis does have precedent in the literature.
In connection with this point, the manuscript could do with more references. Right now, you summarize the literature with a view to support your hypothesis. However, you do not critique methodologies, compare conflicting findings or evaluate robustness. This weakens your manuscript as a review paper. You need to able to state content such as "In name et al., the involvement of molecular pathway X implies that isoform differences may be (necessary but not sufficient) OR (sufficient but not necessary) to cause Y. Or, name et al. found that different isoforms may (or may not) differ in constitutive (or inducible) expression enough to explain Y. Or, name et al. found that - in an unrelated gene - there was no difference in splicing protein X but in their methodology they did not address molecular pathway Y, Or, name et al. found a

similar phenomenon where different splicing protocols or genetics yield different endothelial results…….. Please perform a thorough search of the literature.
3.Tone down causal language. Replace: “explains” with “may explain”, “suggests that X occurs” with “is consistent with”. Please check the entire manuscript for causal or over-reaching claims and tone down.
4.Explicitly state in the limitations section that Explicitly state: that Hypothesis is not yet experimentally validated and Evidence is indirect.

_____

Comment #1
You need alternative explanations, just so readers are aware that the isoform-selective framework may not be the only one operating. Below is a paragraph suggested by chatgpt, which I agree with; that you can insert into the manuscript under a “competing explanations” subsection. Please rewrite in your own words.

Fix
A revised “Alternative Mechanisms” subsection was incorporated into the Discussion to acknowledge competing explanations for the effects of PFKFB3 inhibition. The paragraph was rewritten to reflect the same scientific concepts while avoiding direct reuse of the provided wording. The reviewer is sincerely thanked for providing a clear example section.

Comment #2
The isoform biology is still underdeveloped. You need to present What isoforms exist (even from non-endothelial studies)? Structural differences (N-terminus, localization)? Any functional precedent? You can do this with literature references. It does not need to be exhaustive but must suggest that the isoform hypothesis does have precedent in the literature.

Response
The manuscript was revised to expand the discussion of PFKFB3 isoforms, especially with the subsection titled “Structural and Regulatory Diversity of PFKFB3 Isoforms Supports Context-Dependent Function.” Additional detail was included on known transcript variants, structural differences such as N-terminal regulatory domains, subcellular localization, and functional roles identified in non-endothelial systems. Supporting literature was added to establish precedent for isoform-specific regulation.

Comment #3
In connection with this point, the manuscript could do with more references. Right now, you summarize the literature with a view to support your hypothesis. However, you do not critique methodologies, compare conflicting findings or evaluate robustness. This weakens your manuscript as a review paper. You need to able to state content such as "In name et al., the involvement of molecular pathway X implies that isoform differences may be (necessary but not sufficient) OR (sufficient but not necessary) to cause Y. Or, name et al. found that different isoforms may (or may not) differ in constitutive (or inducible) expression enough to explain Y. Or, name et al. found that - in an unrelated gene - there was no difference in splicing protein X but in their methodology they did not address molecular pathway Y, Or, name et al. found a similar phenomenon where different splicing protocols or genetics yield different endothelial results…..Please perform a thorough search of the literature.

Fix
The manuscript was revised to incorporate additional references and to improve critical evaluation of the literature. Statements were modified to distinguish between correlation and causation, and to acknowledge methodological limitations. Comparative and conditional language was added to reflect whether findings are necessary, sufficient, or context-dependent. These revisions took place in various sections within the Discussion.

Comment #4
Tone down causal language. Replace: “explains” with “may explain”, “suggests that X occurs” with “is consistent with”. Please check the entire manuscript for causal or over-reaching claims and tone down.

Fix
The manuscript was revised throughout to reduce casual language and avoid overinterpretation. Statements were modified to reflect correlation, consistency, or possibility rather than definitive causation. These edits took place across the entire document.

Comment #5

Explicitly state in the limitations section that Explicitly state: that Hypothesis is not yet experimentally validated and Evidence is indirect.
Fix
A limitations section was added to explicitly clarify that the proposed isoform-selective hypothesis has not been directly tested and that current evidence is indirect.

Thank you for addressing my comments. The paper is now acceptable. However, I will need you to upload a doc file (preferably as a libreoffice writer file) of your manuscript. Please format it according to the guidelines here: https://jhss.scholasticahq.com/for-authors, quick guide for submission. Please make sure the references are numbered in the references section and formatted per APA guidelines. Note that a live link is needed for each reference (unless it does not exist).

Thank you for addressing my comments. Accepted.