



Review Article

Regulatory Mechanisms of NKG2DL Expression and Clinical Significance in Hepatocellular Carcinoma Cells



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Abstract

Hepatocellular carcinoma (HCC) is a malignant tumor with high incidence and mortality rates worldwide, in which immune evasion mechanisms play a crucial role in its progression and treatment. Natural killer group 2D ligands (NKG2DL), as key molecules activating immune cells, significantly influence the immune evasion of liver cancer through their regulatory mechanisms. This review summarizes the regulatory mechanisms of NKG2DL expression, including genetic, signaling pathway, non-coding RNA, and stress response modulation, and discusses their expression patterns and clinical relevance in HCC. Studies have shown that the expression status of NKG2DL not only impacts patient prognosis and therapeutic response but also provides potential targets for HCC immunotherapy. Future research should focus on the molecular networks regulating their expression and their synergy with immunotherapy to provide a theoretical basis for developing more precise diagnostic and personalized treatment strategies for HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent and lethal malignancies worldwide. Its early diagnosis is challenging, the tumor exhibits marked biological heterogeneity, the proportion of resectable cases is limited, and responses to conventional chemotherapy and targeted therapies vary among individuals, all of which contribute to suboptimal overall survival.¹ Tumor immune evasion is considered a key driver of HCC onset and progression. In recent years, with the application of immune checkpoint inhibitors (such as programmed cell death 1 (PD-1)/programmed cell death ligand 1 (PD-L1) and cytotoxic T-lymphocyte-associated protein 4 antibodies) in advanced HCC, immunotherapy has made significant strides. However, objective response rates and the durability of responses remain limited, underscoring the importance of further research into the tumor immune microenvironment and innate immune receptor pathways.² Against this backdrop, the Natural killer group 2D (NKG2D) receptor and its ligand axis have attracted considerable attention for their role in bridging innate and adaptive immunity.³

NKG2D is a potent activating receptor broadly expressed on

natural killer (NK) cells, CD8⁺ T cells, as well as subsets of $\gamma\delta$ T cells and innate-like lymphocytes. By recognizing stress-induced molecules, it triggers cytotoxicity and cytokine secretion, thereby mediating antitumor immune responses.⁴ Natural killer group 2D ligands (NKG2DL) in humans primarily include MHC class I chain-related proteins A/B (MICA and MICB) and UL16-binding proteins (ULBPs, ULBP1–6). These molecules are upregulated on the surface of cells under pressures such as viral infection, DNA damage, metabolic stress, or oncogenic transformation. Upon recognition by NKG2D, they rapidly activate effector cells to eliminate abnormal cells.⁵ However, tumor cells employ multiple mechanisms to downregulate or functionally impair NKG2DL expression to evade immune surveillance. These include transcriptional and epigenetic regulation; post-transcriptional repression mediated by microRNAs (miRNAs)/long non-coding RNAs (lncRNAs); modulation of protein folding and trafficking by endoplasmic reticulum stress and autophagy pathways; protease (e.g., ADAM family)-mediated shedding of membrane-bound ligands to generate soluble NKG2DL; and exosomal transport of ligands that desensitizes NKG2D receptors—collectively weakening the effector functions of NK and CD8⁺ T cells.⁶ This review aims to systematically delineate the expression landscape and regulatory networks of NKG2DL in HCC, elucidate their roles in immune evasion and variability in immunotherapy responses, and assess their clinical translational potential as biomarkers and therapeutic targets, including inhibiting shedding, blocking soluble ligands, upregulating membrane-bound ligands, and engineering cell therapies targeting NKG2DL. A deeper understanding of these mechanisms is expected to provide a theoretical basis and new avenues

Keywords: Hepatocellular carcinoma; Natural killer group 2D ligand; NKG2DL; Immune evasion; Signaling pathway; Non-coding RNA; Immunotherapy.

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for developing more precise and durable immunotherapeutic strategies for HCC.

The biological background of NKG2D and its ligands

The NKG2D receptor is mainly expressed on the surface of NK cells, CD8⁺ T cells, $\gamma\delta$ T cells, and other immune cells. Its core function is to recognize and bind ligands expressed on the surface of stressed cells, thereby mediating the activation of immune cells and the killing of target cells.⁴ NKG2D and its ligands play important roles in immune surveillance, antitumor responses, and antiviral infections, and are key components of the innate immune system.

NKG2DL belongs to a diverse family of molecules, mainly including MICA, MICB, and ULBP family members (ULBP1–6). These ligands are usually not expressed or are only expressed at low levels on healthy cells, but are significantly upregulated in damaged, stressed, infected, or cancerous cells.⁶ Therefore, the NKG2D receptor can induce immune cells to clear abnormal cells by recognizing these ligands.⁷

The expression of NKG2DL is regulated by multiple factors, including gene transcription, epigenetic modifications, and cellular stress responses. For example, DNA damage, oxidative stress, infection, and inflammatory cytokines (such as interferon-gamma and tumor necrosis factor-alpha) can all induce the upregulation of these ligands, thereby enhancing the recognition and elimination of abnormal cells by immune cells.⁸ In addition, the interaction between NKG2D and NKG2DL not only activates the cytotoxic function of NK cells but also enhances the antigen-specific killing ability of CD8⁺ T cells.⁹

However, many tumor cells downregulate the expression of NKG2DL through various mechanisms to evade immune system attacks. These mechanisms include epigenetic modifications (such as DNA methylation and histone deacetylation) and the secretion of soluble ligands to block the recognition of the NKG2D receptor.¹⁰ In CD8⁺ T cells, NKG2D typically does not trigger a cytolytic response because it signals solely through Dap10, which provides costimulatory signals. However, in senescent CD8⁺ T cells, NKG2D can trigger cytolysis via a sestrin-based interaction.¹¹ A deeper understanding of these regulatory mechanisms is of great significance for the development of new tumor immunotherapy strategies.

Regulatory mechanisms of NKG2DL expression in HCC

Gene-level regulation

The expression of NKG2DL is regulated at the gene level through various mechanisms, mainly involving transcriptional activation, transcriptional repression, and post-transcriptional processing and regulation.¹² In HCC, specific transcription factors (such as nuclear factor kappa B (NF- κ B) and activator protein 1 (AP-1)) can bind to the promoter regions of NKG2DL genes to activate their transcription, thereby promoting ligand expression.¹³ Conversely, certain repressive transcription factors (such as p53) can inhibit the transcription of these genes, reducing ligand expression on the cell surface and weakening the recognition ability of immune cells.¹⁴

Epigenetic regulation of genes also plays a key role in the expression of NKG2DL. DNA methylation and histone modifications are important epigenetic factors affecting NKG2DL gene expression.^{15,16} Studies have shown that when the promoter region of the NKG2DL gene is highly methylated, the transcription level of the ligand is significantly reduced, leading to tumor cells evad-

ing immune surveillance.⁷ In addition, histone acetylation and deacetylation modifications also play important regulatory roles in ligand gene expression.¹⁷ Histone acetylation is usually associated with gene activation, while deacetylation is associated with gene silencing. These modifications affect the binding efficiency of transcription factors by altering chromatin structure.¹⁸

By understanding these gene-level regulatory mechanisms, we can further reveal how HCC downregulates the expression of NKG2DL to evade immune surveillance, thereby providing a basis for the development of new immunotherapy strategies.

Signal pathway regulation

The expression of NKG2DL is also regulated by various cellular signaling pathways, which play important roles in the stress response and immune evasion of HCC.¹⁹

Cytokine signaling pathways play a key role in regulating the expression of NKG2DL. For example, pro-inflammatory cytokines such as interferon-gamma and tumor necrosis factor-alpha promote the upregulation of NKG2DL by activating the JAK/STAT or NF- κ B signaling pathways.²⁰ These cytokines are usually significantly increased during inflammatory responses, thereby enhancing the recognition and killing of tumor cells by immune cells.

In addition, the MAPK/ERK signaling pathway also plays a role in the regulation of NKG2DL expression.²¹ This pathway is activated under stress conditions and can promote the expression of many stress-related genes, including NKG2DL. Especially under conditions of oxidative stress and DNA damage, the activation of the MAPK signaling pathway helps to enhance the expression of NKG2DL on the surface of tumor cells, thereby improving the effectiveness of tumor immune surveillance.²²

The PI3K/Akt signaling pathway is usually associated with the NKG2DL expression.²³ This pathway is abnormally activated in many tumors, promoting cell proliferation and survival,²⁴ and helping tumor cells evade immune surveillance by downregulating NKG2DL expression. The PI3K/Akt pathway regulates NKG2DL gene expression and its presentation on the cell surface by regulating downstream molecules such as mTOR, thereby reducing the recognition efficiency of immune cells.²⁵

The Wnt/ β -catenin signaling pathway is also believed to be associated with the suppression of NKG2DL expression in some tumors. The accumulation of β -catenin can inhibit the expression of NKG2DL, thereby weakening the activation of immune cells and their killing of tumor cells.²⁶ Abnormal activation of this signaling pathway has been reported in many cancers and is one of the important mechanisms by which tumor cells evade the immune system.

By understanding the regulatory mechanisms of these signaling pathways, we can better reveal how HCC regulates the expression of NKG2DL through complex signaling networks to evade immune surveillance. This provides a new research direction for the development of antitumor immunotherapy strategies targeting signaling pathways in the future.

Regulation by non-coding RNA (ncRNA)

ncRNA plays an important role in the regulation of NKG2DL expression, mainly including miRNAs, lncRNAs, and circular RNAs (circRNAs).²⁷

miRNAs are a class of small RNAs that inhibit gene translation or accelerate messenger (mRNA) degradation by binding to the 3' untranslated region of target gene mRNA, thereby regulating the expression of NKG2DL.²⁸ For example, certain miRNAs (such as miR-20a and miR-93) have been found to downregulate the expression of NKG2DLs such as MICA and MICB, thereby helping

tumor cells evade immune surveillance.^{29,30}

lncRNAs regulate the expression of NKG2DL through various mechanisms, including interacting with chromatin remodeling factors, affecting the recruitment of transcription factors, and competitively binding miRNAs.³¹ Some lncRNAs act as “sponges” to competitively bind miRNAs, reducing the inhibitory effect of miRNAs on NKG2DL mRNA and thereby upregulating the expression of NKG2DL.³² Conversely, some lncRNAs promote the expression of inhibitory miRNAs, thereby downregulating the expression of NKG2DL and helping tumor cells achieve immune evasion.³³

In addition, circRNAs, a class of ncRNAs with a circular structure, usually have a “sponge” effect on miRNA regulation.³⁴ In HCC, certain circRNAs bind to specific miRNAs, thereby relieving the inhibitory effect of these miRNAs on NKG2DL, ultimately promoting the expression of NKG2DL. This circRNA-miRNA-mRNA interaction network plays an important regulatory role in the regulation of NKG2DL.³⁵

The complex regulatory network of ncRNA provides a new understanding of how tumor cells downregulate the expression of NKG2DL to evade immune surveillance. In-depth research on the role of ncRNA in the expression of NKG2DL will not only help reveal the mechanisms of HCC immune evasion but also provide a potential new direction for immunotherapy targeting ncRNA.

Regulation by exogenous and endogenous stress factors

Exogenous and endogenous stress factors also play important roles in the regulation of NKG2DL expression.³⁶

Exogenous stress factors include chemotherapy drugs, radiotherapy, and viral infections, which can promote the expression of NKG2DL by inducing DNA damage and oxidative stress within cells. For example, certain chemotherapy drugs (such as doxorubicin and cisplatin) can induce DNA damage and increase reactive oxygen species levels, activating related stress response pathways and thereby upregulating the expression of NKG2DL, enhancing the sensitivity of tumor cells to immune cells.^{37,38}

Endogenous stress factors mainly refer to intracellular metabolic disorders, oxidative stress, and DNA damage. These endogenous stress signals are usually triggered by abnormal cell proliferation and metabolic activities.³⁹ In HCC, overactive metabolic activities and oxidative stress activate stress response-related signaling pathways, such as the MAPK/ERK and ATM/ATR pathways, thereby upregulating the expression of NKG2DL.⁴⁰ In addition, intracellular oxidative stress can also promote the transcriptional activation of NKG2DL genes by regulating the activity of transcription factors (such as NF- κ B and AP-1).¹³

However, tumor cells may also regulate these stress responses to evade immune system surveillance. For example, regulating the expression of antioxidant genes reduces intracellular oxidative stress levels, thereby reducing the expression of NKG2DL and weakening the killing effect of immune cells.⁴¹ This mechanism of regulating NKG2DL expression through stress responses provides tumor cells with an additional means of immune evasion.

In-depth research on the regulatory effects of exogenous and endogenous stress factors on NKG2DL will help reveal how HCC regulates immune surveillance by modulating stress responses and provide new ideas for the development of novel anticancer therapies.

Expression patterns and clinical relevance of NKG2DL in HCC

The expression patterns of NKG2DL in HCC are highly hetero-

geneous, reflecting the complexity of the tumor immune micro-environment. Its correlation with patient prognosis and treatment response is significant, and it has important clinical application potential.⁴² In patients with HCC, the expression levels of NK-G2DL (such as MICA, MICB, and the ULBP family) are usually influenced by both intrinsic tumor gene regulation and external microenvironmental factors.⁴³ Cullinan Therapeutics' clinical trial targeting MICA/B aimed to restore NKG2D-mediated immune recognition in solid tumors with marked heterogeneity and immune tolerance (particularly HCC) by inhibiting shedding, stabilizing membrane-bound expression, and leveraging Fc engineering to enhance effector cell activity. In addition, the trial incorporated standardized companion diagnostics and combination therapy strategies to broaden the therapeutic window and improve translational potential.⁴⁴

In early-stage HCC patients, NKG2DL mainly exists in a membrane-bound form, which can effectively activate NK cells and cytotoxic T lymphocytes to inhibit tumor growth and spread.⁴⁵ However, in advanced HCC, tumor cells weaken ligand membrane expression through protease-mediated cleavage or endocytosis, leading to the release of soluble ligands (such as sMICA), which attenuate the activity of the NKG2D receptor and promote immune evasion.⁴⁶

This expression pattern is not only significantly correlated with the prognosis of HCC patients but also plays a key role in treatment response. Studies have shown that high expression of NKG2DL is associated with better patient survival rates, while elevated levels of soluble ligands in serum are closely related to poorer prognosis and higher recurrence risk.⁴⁷ In addition, in immunotherapy with immune checkpoint inhibitors (such as PD-1/PD-L1 inhibitors), the expression status of NKG2DL may modulate treatment efficacy.⁴⁸ High levels of ligand membrane expression can enhance the effectiveness of immune checkpoint inhibitors, while the presence of soluble ligands may weaken the immune response, suggesting the need to develop blocking strategies targeting soluble ligands to improve therapeutic outcomes.

Based on its significant clinical relevance, NKG2DL has the potential to serve as a diagnostic and prognostic biomarker for HCC. The membrane expression level of the ligand can be used to assess tumor immune activity, while the detection of soluble ligands in serum can serve as a reference indicator for monitoring disease progression and treatment response. At the same time, therapeutic strategies targeting the regulation of NKG2DL expression (such as blocking sMICA or enhancing the stability of membrane ligands) are becoming a research hotspot for novel HCC immunotherapy. In the future, further dissection of the expression patterns of NK-G2DL and its relationship with immunotherapy through multi-omics integration and clinical research will help promote its application in precision medicine and provide more personalized treatment options for HCC patients.

Application potential of NKG2DL in HCC immunotherapy

NKG2DL holds significant application potential in HCC immunotherapy. Targeting its regulation not only enhances the effects of existing treatment strategies but also provides new directions for combination therapies. By modulating the expression and function of NKG2DL, antitumor immune responses can be significantly boosted. For example, restoring the stable membrane expression of NKG2DL on tumor cells and inhibiting the production of soluble ligands (such as sMICA) by suppressing protease activity can re-activate the effector functions of NK cells and cytotoxic T lymphocytes.

phocytes, thereby enhancing immune surveillance. In addition, some experimental studies have explored the use of small-molecule drugs or gene editing technologies (such as CRISPR/Cas9) to regulate the gene expression of NKG2DL, achieving preliminary success,⁴⁹ which lays the foundation for the development of novel immunotherapy strategies in the future.

Targeted therapies against NKG2DL have become a research hotspot. Monoclonal antibodies and fusion proteins prevent the binding of soluble ligands to the NKG2D receptor, thereby preventing receptor downregulation and functional inactivation. For example, sMICA-specific antibodies have shown significant antitumor effects *in vitro* and animal models.⁵ In addition, Celyad Oncology is exploring the safety and feasibility of broad-spectrum solid tumor chimeric antigen receptor T-cell therapies through strategies such as leveraging the native NKG2D receptor and allogeneic engineering, while Leucid Bio employs its “Lateral CAR” geometric optimization platform to enhance immunological synapse efficiency and durability in solid tumors. Both approaches have been evaluated in humans and have demonstrated manageable safety profiles and early biological activity.⁵⁰ The combination of NKG2DL regulation with other immunotherapeutic approaches has broad prospects. Combined with immune checkpoint inhibitors (such as PD-1/PD-L1 inhibitors), NKG2DL regulation can compensate for the insufficient efficacy of checkpoint inhibitors in some patients and further enhance antitumor immune activity.⁴⁸ In addition, the combination with tumor vaccines or cytokine therapies (such as interleukin-2, interleukin-15) can improve treatment outcomes through synergistic mechanisms. Studies have also shown that combining ligand regulation strategies with oncolytic virus therapy has the potential to break through the current resistance bottlenecks in HCC immunotherapy.⁵¹

In summary, the application potential of NKG2DL in HCC immunotherapy is enormous. Its regulation not only provides targets for monotherapy but also plays an important role in combination therapies. In the future, further development of more efficient treatment strategies through in-depth basic research and clinical validation will provide more precise and personalized immunotherapy options for HCC patients.

An NKG2D–NKG2DL-centered integrative strategy for HCC treatment

In HCC, tumor cells can upregulate membrane-bound NKG2D ligands (MICA/MICB and the ULBP family) under oxidative stress, DNA damage, and inflammatory stimuli, thereby enhancing NKG2D-mediated immune surveillance by NK and CD8⁺ T cells.⁵² Conversely, metalloproteases such as ADAM10/17 can shed these membrane ligands into soluble forms (sMICA/sMICB), leading to NKG2D receptor downregulation, effector cell desensitization, and immune evasion.⁵³ Accordingly, key intervention goals include: (i) increasing membrane-bound NKG2DL expression on tumor cells, (ii) inhibiting aberrant shedding and exosomal trafficking, and (iii) remodeling the immune microenvironment to protect or enhance effector cell function.

Current preclinical evidence indicates that multiple small molecules and pathway-directed interventions can achieve these goals: by inhibiting chronic inflammation circuits (STAT3 and NF- κ B), modulating MAPK/ERK and PI3K/Akt/Wnt- β -catenin signaling, and leveraging epigenetic mechanisms (HDAC/DNMT) and miRNA network remodeling, membrane NKG2DL expression on HCC cells can be upregulated, accompanied by increased NK-cell infiltration and cytotoxicity.^{54–58} In parallel, certain strategies can

indirectly reduce ADAM10/17 activity and oxidative stress imbalance, thereby decreasing sMICA release and extending the tumor’s “immune recognizability window”.⁵ Clinically, in exploratory combinations with TACE, radiotherapy, or PD-1/PD-L1 inhibitors, signals of improved NK functional metrics and higher disease control rates have been observed, suggesting potential synergy with the NKG2D axis through increased tumor immunogenicity and reduced immunosuppression.

For translation, standardized measurements and companion diagnostics should be prioritized: (1) establish a pharmacodynamic fingerprint centered on membrane MICA/MICB/ULBPs, serum sMICA, ADAM10/17 activity, and NK functional scores; (2) use gene editing and pharmacologic blockade to validate the causal chain of “regulatory factor—signaling pathway—NKG2DL—effector cells,” complemented by single-cell and spatial omics to delineate the immune ecosystem; and (3) design stratified combination trials with immune checkpoint inhibitors, oncolytic viruses, or NKG2D CAR NK/T therapies to build a reproducible integrative immunotherapy pathway. The overarching objective is not merely to “upregulate ligands” but to achieve systemic immune rebalancing, elevating membrane-bound ligands, reducing soluble ligands, alleviating chronic inflammation, and enhancing effector cytotoxicity, through multi-target remodeling of immune metabolism and epigenetics.

Discussion

Integrated evidence indicates that the NKG2D–NKG2DL axis in HCC is not only a central hub bridging innate and adaptive immunity but also a “sensitization fulcrum” linking radiotherapy/intervention, targeted therapy, and immune checkpoint blockade. On one hand, oxidative stress, DNA damage response (DDR), and inflammatory signals induce membrane expression of MICA/MICB and the ULBP family, transiently enhancing tumor “visibility.” On the other hand, ADAM10/17-mediated shedding, exosomal trafficking, epigenetic repression, and ncRNA networks collectively reduce membrane-bound ligands while increasing sMICA/sMICB, leading to NKG2D desensitization and immune escape. This dynamic “induction–shedding–desensitization” interplay explains the heterogeneity of NKG2DL measurements, immune responses, and prognosis in HCC, and suggests that strategies aiming solely to upregulate ligands are unlikely to achieve durable benefit. Clinically, the NKG2DL state interacts with systemic therapies in ways that can be leveraged: radiotherapy/intervention and certain chemotherapies can create a DDR-driven “ligand visibility window”; anti-VEGF/multitarget tyrosine kinase inhibitors may improve ligand presentation and immune-cell infiltration through vascular normalization and stress-response modulation; ICIs can amplify downstream NKG2D effects once inhibition is lifted; and inhibiting ADAM10/17 or exosome biogenesis may reduce soluble ligands and receptor desensitization. Engineered cell therapies (e.g., NKG2D-CAR NK/T) and bispecific molecules can serve as “residual disease clearance/consolidation” modalities after response but should be guided by ligand “accessibility” rather than “total amount” to minimize bystander injury.

Based on current evidence, we advocate a timing-first, rhythmic strategy to integrate the NKG2D–NKG2DL axis: first, induce a membrane MICA/MICB/ULBP “visibility window” with radiotherapy/DDR or short-course epigenetic–metabolic modulation; then layer PD-1/PD-L1 $\pm$ anti-VEGF during the window to amplify effects; finally, consolidate clearance with NKG2D-directed cell therapies/bispecifics. To enhance predictability and operability, we

propose a “ligand accessibility–receptor functionality” composite score, incorporating membrane ligand quantitation, sMICA/sMICB, ADAM10/17, and exosome metrics, and NKG2D–DAP10 signaling to guide population stratification and temporal adjustments. In patients with marginal liver function or advanced fibrosis, prioritize local delivery and short-pulse induction and explore combined blockade of “shedding–exosome” pathways to reduce desensitization and soluble ligand burden. Single-cell and spatial omics can serve as the technological backbone for companion diagnostics and real-time pharmacodynamic fingerprinting, supporting validation and optimization of the above scoring system and rhythmic dosing in prospective trials.

Outlook

Future research directions should focus on the following breakthroughs: First, use multi-omics technologies (such as transcriptomics, epigenomics, and proteomics) to comprehensively dissect the molecular networks regulating NKG2DL expression, especially exploring the expression heterogeneity in different stages and subtypes of HCC. Second, based on the dynamic changes in the tumor immune microenvironment, develop intervention strategies to restore or enhance NKG2DL function, such as blocking the production of soluble ligands or promoting the stable expression of membrane ligands. In addition, combining single-cell sequencing and spatial transcriptomics technologies can more precisely map the distribution of NKG2DL in tumor tissues and its interactions with immune cells.

To enhance the clinical application value of NKG2DL, future efforts should focus on translational research in diagnosis, prognosis assessment, and treatment. For example, develop serum biomarker detection methods based on NKG2DL for non-invasive early diagnosis of HCC; optimize therapeutic strategies targeting NKG2DL, including antibody therapies, small molecule inhibitors, and ligand modification technologies; explore the combination of NKG2DL regulation strategies with immune checkpoint inhibitors, oncolytic viruses, or cell therapies to further enhance therapeutic efficacy. In addition, establishing large-scale, multi-center clinical research platforms to verify the reliability and universality of NKG2DL as a biomarker and therapeutic target is also an important task for the future.

Conclusions

Research on NKG2DL not only has important theoretical significance in the immune regulation of HCC but also provides a new perspective and strategy for clinical immunotherapy.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Study concept and design (CW, WM), literature search (QG), analysis and interpretation of data (QG), drafting of the manuscript (QG), preparation of figures (CW), and supervision (CW, WM). All authors have read and approved the final version of the manuscript.

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