Abstract. Most tumor cells still exhibit active glucose uptake and glycolysis under aerobic conditions, a phenomenon known as the Warburg effect or aerobic glycolysis. Pyruvate kinase, one of the key enzymes in the cell glycolysis pathway, can promote the conversion of glucose to pyruvate and produce energy. Pyruvate kinase M2 (PKM2), a competitive PK subtype, is an important regulator of the aerobic glycolysis pathway in tumor cells and plays a direct role in gene expression and cell cycle regulation. Human papillomavirus (HPV) persistence is the main risk factor for cervical cancer. In recent years, it has been discovered that HPV plays an important role in malignant anal tumors and oral cancer. HPV oncoprotein E7 can promote the Warburg effect and produce a large amount of ATP, which may meet the energy requirements of cancer cell division. There appears to be a regulatory relationship between HPV E7 and PKM2, but the specific mechanism is mostly unknown. The present review article discusses the role of HPV E7 in transcriptional regulation, enzyme activity regulation, protein kinase activity regulation, post-translational modification and the immune microenvironment of PKM2 in the occurrence and development of cervical cancer.

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1. Background

The energy of cells mainly comes from glycolysis and the oxidative phosphorylation of glucose, where the former produces pyruvate. It has long been hypothesized that pyruvate is metabolized by mitochondrial oxidative phosphorylation to produce water and carbon dioxide under aerobic conditions, while it is converted to lactic acid by lactate dehydrogenase under anaerobic conditions during glycolysis. However, in the 1920s, German physiologist Otto Warburg reported that the main source of energy acquisition of tumor cells is anaerobic glycolysis of sugar, where the sugar undergoes aerobic glycolysis and oxidative phosphorylation even when the oxygen supply is sufficient (1,2). This is known as the Warburg effect. This switching from oxidative phosphorylation to glycolysis is considered to be a major feature of tumors (3,4). Glycolysis is an oxygen-independent process in which the rate-limiting step is controlled by a group of enzymes, including phosphofructokinase, hexokinase, glucokinase and pyruvate kinase (PK). Competitive PK is an important regulatory protein involved in glucose catabolism (4,5-7), and there are several different
isoforms of PK in mammals, which differ in allosteric regulation and tissue expression. Cancer cells tend to preferentially express a specific competitive PK subtype, PKM2, which plays an important role in tumor metabolism (8-10).

Previous studies have shown that human pathogenic viruses have a reprogramming effect on tumor metabolism (11,12). In some cases, the interaction between viral proteins and cell proteins leads to the malignant transformation of cells, and the metabolism changes accordingly to meet the energy needed for the rapid proliferation of these tumor cells (13,14). For example, HPV E7 oncoprotein can interact with SMAD2/3/4 and cause SMAD3 suppression, which results in TGF-β signaling pathway inhibition (15). Studies have shown that PKM2 is highly expressed in embryonic and tumor cells, and the role of PKM2 in cervical cancer has been a long-term concern (16-18). Since the beginning of the 21st century, it has been known that PKM2 plays an important role in the metabolism of cervical cancer cells (19,20), and the function of its non-metabolic pathway has also been studied recently (21,22).

As one of the most common types of cancer threatening women's health (23), cervical cancer has always had global attention, and human papillomavirus (HPV) persistence is the main risk factor for cervical cancer (24). However, the specific regulatory mechanism of PKM2 and HPV is not clear.

At present, >200 types of HPV have been identified (25), and 12 HPV types have been defined as high-risk carcinogenic types by the World Health Organization (WHO), namely, HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (26). HPV belongs to the papillomaviridae family, which is a family of viruses that are unenveloped and contain a double-stranded DNA genome. The genetic material of the virus is surrounded by icosahedral capsids, which are composed of structural proteins L1 and L2. The L1 genome sequence encodes the main proteins leads to the malignant transformation of cells, and the conversion of PEP and ADP into pyruvate and ATP, and also as a dimer with low affinity for PEP, enhancing the enzymatic activity (36). It has been shown that PKM2 not only plays a central role in metabolic reprogramming, but also plays a direct regulatory role in gene expression and subsequent cell cycle processes (37).

As a glycolytic enzyme, PKM2 is mainly located in the cytoplasm; however, PKM2 in tumor cells accumulates in the nucleus (34). Monomeric PKM2 translocates to the nucleus, acts as a histone kinase and upregulates the expression of proto-oncogene c-Myc, thus promoting the Warburg effect (38). In addition, the deacetylation of residue K62 of PKM2 can promote the transport of PKM2 to the nucleus and binding to β-catenin, thus promoting the transcription of the cyclin D1 gene and the process of the cell cycle (39). Therefore, intranuclear PKM2 is essential for tumorigenesis. Moreover, the direct interaction between lncRNA-AC020978 and PKM2 in non-small cell lung cancer can enhance the stability of PKM2 (40). Furthermore, lncRNA-AC020978 can promote the nuclear translocation of PKM2 and regulate the transcriptional activity of hypoxia-inducible factor α (HIF-1α) enhanced by PKM2 (40).

It has been reported that the epidermal growth factor receptor (EGFR)-mitogen-activated protein kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway phosphorylates PKM2 at S37 and promotes nuclear translocation in hepatocellular carcinoma cells stimulated by EGF (41). It has also been observed that HPV16 E7 can promote the acetylation of K433 of PKM2 (42). This acetylation can in turn promote the kinase activity and nuclear localization of PKM2. Thus, it can be suggested that E7 plays a direct role in the nuclear localization of PKM2, but this needs further exploration.
3. Transcriptional regulation of PKM2 by E7

The expression of PKM2 is driven by several cellular signaling pathways, including transcription factor 1 (SP1), HIF-1α (43), mammalian target of rapamycin (mTOR) (44), c-Myc (45), nuclear factor κB (NF-κB) (46) and peroxisome proliferator-activated receptor γ (PPARγ) (47). The promoter of PKM2 is composed of three cis-acting regions and three GC boxes (48). In previous research, five possible binding sites of SP1 and SP3 were found in the PKM2 promoter. SP1 was shown to activate the transcription of the PKM2 gene structurally by binding to the common DNA-binding site (GC box) in the promoter of the PKM gene. SP3 cooperated with SP1 to enhance the expression of PKM2 (49). The expression of E6 and E7, and the downregulation of phosphatase and tensin homolog on chromosome ten (PTEN) and thioredoxin interactions protein significantly promoted glucose transporter protein 1, glucose uptake (50) and the dephosphorylation of SP1, which in turn promoted the expression of PKM2 (51). In addition, the protein and mRNA expression levels of liver kinase B1 (LKB1) were downregulated by HPV16 E6/E7, while the deletion of LKB1 upregulated the expression and activity of SP1. SP1 further upregulated the expression of genomic amplification of human telomerase gene (hTERC) at the mRNA and gene amplification levels. Based on this, the HPV-LKB1-SP1-hTERC axis was proposed (52). The aforementioned studies demonstrated that E7 can not only enhance the activity of SP1 by enhancing the absorption of glucose, but also by downregulating LKB1 to increase the activity of SP1 and ultimately promote the expression of PKM2 (Fig. 1).

PKM2 interacts with HIF-1α and promotes its function (30). HIF-1α is a common regulatory component of cellular metabolism and plays a role in regulating the function of immune cell effectors. The binding of HPV E7 to PKM2 can promote the dissociation of PKM2 tetramers to form inactivated dimers, and the latter can stimulate the activation of HIF-1α, which in turn activates the mTOR signaling pathway and inhibits the autophagy of cancer cells (53). Previous studies have shown that mutation of exon 10 of the PKM gene promotes the translocation of PKM2 to the nucleus, which is considered to be related to the increase in HIF-1α activity and may play an important role in tumor metabolism (54,55). There is also evidence that ribonucleotide reductase regulatory subunit M2 (RRM2), considered a new downstream target of HPV E7, is upregulated at the transcriptional level through E7-pRb interaction and the binding of E2F to the RRM2 promoter (56). The overexpression of RRM2 enhances the expression of HIF-1α and VEGF by activating the ERK1/2 signaling pathway in cervical cancer cells and is significantly associated with the increase of microvessel density in cervical cancer tissues (56). It is worth noting that PKM2 can interact with the HIF-1α transcriptional complex to regulate its transcription through a positive feedback loop, to promote the occurrence and development of tumors (Fig. 1) (57,58).

When PI3K binds to growth factor receptors (such as EGF-R) in the PI3K/AKT/mTOR network, the structure of AKT changes and is activated. This in turn phosphorylates and activates or inhibits the activity of a series of downstream substrates (such as apoptosis-related proteins Bad and Caspase 9), thus regulating cell proliferation, differentiation, apoptosis and migration (59-61). In hypoxic and normoxic HPV-positive cancer cells, the PI3K/AKT/mTOR network plays a key role in the virus-host cell interface (62). Under hypoxic conditions, HIF-1α induces the activation of the PI3K/AKT/mTOR signal pathway and regulates PKM2 by downregulating the expression of c-myc and upregulating heterogeneous ribonucleoproteins (hnRNPs) (63). In hypoxic HPV-positive cancer cells, hypoxia can block the effect of E6/E7, which is canceled by AKT and a high glucose supply (64). It is worth noting that the ability of E7 to upregulate the activity of AKT depends on its ability to bind to and inactivate pRb. It has been observed that knockout of pRb with shRNA alone is sufficient to activate AKT activity in differentiated keratinocytes (65). In addition, E7 may participate in the PI3K/AKT/SGK signaling pathway by activating the phosphorylation of the AKT S473 site (Fig. 1) (66).

A study by Yang et al (67) showed that under normoxic conditions, PKM2 is transcriptionally upregulated by growth factors. In addition, the upregulation of PKM2 induced by EGF depends on the activation of the PLCRINCK1/γ/IKKβ/RELA signaling cascade. The activation of EGFR leads to an increase in glucose uptake and lactic acid production, which may be dependent on PKM2 expression. Previous studies have shown that the change in EGFR level is related to the change in cell proliferation in vitro. There is a correlation between the level of E6/E7 mRNA and the level of EGFR protein in cervical cancer cells, and E6/E7 may change the level of EGFR through the Rb pathway (68). Moreover, NF-κB-activated PKM2 plays an important role in EGFR-induced pleomorphic glioblastoma cell metabolism and brain tumor growth (67). NF-κB is a transcription factor that was first discovered in the nuclear extract of B lymphocytes in 1986, and can specifically bind to the enhancer B sequence of the immunoglobulin κ light chain gene and promote its expression (69). NF-κB has a function in inhibiting apoptosis, which is closely related to numerous processes such as tumorigenesis, growth and metastasis (70). In addition, HPV E6 and E7 are important regulatory proteins in cervical cancer, which are closely related to NF-κB transcriptional activity in host cells (71), and drive chronic inflammation in cancer development (72,73).

PPARγ plays an important role in the transcription of PKM2 isozyme genes (47,72,73). PPARγ is highly expressed in adipose tissue, and is also expressed in vascular parietal cells (such as monocytes, macrophages, endothelial cells and smooth muscle cells) and cardiomyocytes (74). The activation of AKT is known to promote the association between PPARγ and its response elements, and promotes the occurrence of liver malignancy in fatty liver cells with a PTEN deletion (75). By contrast, the expression of PPARγ can be inhibited by HPV16 E7, which promotes the proliferation and invasion of cervical cancer cells (76).

In summary, SP1, HIF-1α, NF-κB and PPARγ can regulate PKM2 in tumor cells and interact with HPV E7, which indicates that E7 may play a key role in PKM2 transcription (Fig. 1).

4. Selective splicing control of PKM2 precursor mRNA by E7

Previously, David et al (57) revealed the existence of hnRNP, polypyrimidine bundle binding proteins [PTB] also known as hnRNPl, hnRNPA1 and hnRNPA2 in tissues by studying the
alternative splicing molecular mechanism of PKM2 isoforms. hnRNP1, hnRNP2 and PTB can be mediated by c-Myc (57), which is an essential transcription factor that regulates the expression of numerous genes involved in cell growth, proliferation and metabolism. hnRNPs selectively bind to sequences either side of exon 9 of PKM pre-mRNA and inhibit its fusion with mature mRNA, thus indirectly promoting the fusion of exon 10 and mature mRNA, and resulting in the expression of PKM2 mRNA in cancer cells (77). This mechanism ensures a high PKM2/PKM1 ratio, which helps to promote aerobic glycolysis and provides an advantage for tumorigenesis (77).

In addition, the activation of β-catenin enhances the expression of c-Myc, thus promoting the Warburg effect (78). It has been reported that HPV16 can regulate the migration of β-catenin in HPV-associated oropharyngeal squamous cell carcinoma (78). Furthermore, both E6 and E7 were able to upregulate β-catenin and enhance thymocyte transcription factor-mediated transcription in HPV16-positive oropharyngeal carcinoma cells (79). This evidence suggests that E7 may enhance the expression of c-Myc through β-catenin, thus also regulating PKM2. However, both low-risk and high-risk HPV E7 in cervical cancer cells can interact with c-Myc, but only the interaction between high-risk HPV E7 and c-Myc can functionally enhance the transcriptional activation activity of c-Myc (80).

5. Post-translational modification of PKM2 by E7

Post-translational modification of amino acids endows proteins with other biochemical groups that adapt protein function by changing the properties of amino acids and/or by causing structural changes (such as the establishment of disulfide bonds). These modifications include phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, lipidation and proteolysis (81).

It has been discovered that PKM2 is phosphorylated by a variety of tyrosine kinases and forms dimers to promote the growth of cancer cells (10,82,83). The phosphorylation of PKM2 is common in human cancer and can be increased by HPV16 E7 (4,21,84). Phosphorylated PKM2 Y105F mutants are highly expressed in cancer cells, which can promote cell proliferation and tumorigenesis (4). A recent study has shown that highly upregulated gene in liver cancer (the most upregulated gene in HCC, which was characterized as a novel mRNA-like ncRNA) acts as an adaptor molecule to enhance the binding of lactate dehydrogenase A and PKM2 to fibroblast growth factor receptor 1, increasing the phosphorylation of these two enzymes and thus promoting glycolysis (85). EGFR-activated ERK2 directly binds to PKM2 and phosphorylates S37, but does not phosphorylate PKM1. Phosphorylated PKM2 recruits peptidylprolyl cis/trans isomerase for isomerization of PKM2, thus promoting the binding of PKM2 to the input protein α5 and translocation to the nucleus (86). This nuclear localization of PKM2 further promotes the Warburg effect (Fig. 1).

A high concentration of reactive oxygen species (ROS) can destroy cell composition and damage cell vitality (87). Therefore, controlling the concentration of intracellular ROS is very important for cell proliferation and survival. The sharp increase of intracellular ROS leads to the oxidation of PKM2 at C358, thus inhibiting the activity of PKM2 (88). The cell then transfers from the glucose pathway to the pentose phosphate pathway, resulting in a reduction potential sufficient to detoxify...
ROS. Studies have shown that E7 can induce the accumulation of intracellular ROS (89) by inducing the upregulation of RRM2 and promoting the occurrence of cervical cancer through angiogenesis induced by ROS/ERK1/2/HIF-1α/VEGF (56).

The addition of N-acetylglucosamine (GlcNAc) by O-linked GlcNAc transferase (OGT) is a common PKM2 modification, which participates in the transformation of PKM2 from tetramer to dimer (90). It is reported that HPV can significantly increase the levels of O-linked GlcNAcylation by OGT in cervical tumors. Mouse embryonic fibroblasts transformed with HPV16 E6/E7 experience an upregulation of the mRNA and protein levels of OGT, which promotes cell proliferation and reduces cell senescence (91) (Fig. 1). In addition, acetylation of PKM2 at K433 can inhibit fructose-1,6-bisphosphate (FBP) binding to prevent the dimer-to-tetramer transition of PKM2. HPV16 E6 also enhances the binding of PKM2 with p300 (an acetyltransferase), providing a molecular basis for the E7-stimulated K433 acetylation, which then inhibits PKM2 tetramerization (91).

Due to the accumulation of research on the post-translational modification of PKM2, the non-metabolic effect of PKM2 on tumor cells has been gradually revealed, and thus the mechanism of E7 on PKM2 will open a new chapter.

6. Enzymatic activity regulation of PKM2 by E7

PKM2 is also a phosphorylated tyrosine-binding protein (82). When cells are stimulated by certain growth factors, PKM2 is regulated by phosphotyrosine signaling, which in turn converts glucose metabolites from energy production to anabolic metabolism (82). This is crucial for the rapid growth of cancer cells. It is worth remembering that PKM2 mainly exists in a dimeric form with low activity in tumor cells, while tetramers have a high affinity for substrate PEP. FBP is the intermediate product of glycolysis and the allosteric activator of PKM2, which seems contradictory, but the exchange of dimer and tetramer of PKM2 is very dynamic (92).

It has been revealed that E7 may mimic the effect of inhibitory amino acids (such as alanine or leucine) on PKM2 activity (93). In a high glycolytic NIH3T3 cell line, E7 led to the transformation of PKM2 into its dimeric form and resulted in a decrease in the cellular PK mass action ratio, the glycolysis flux rate and the (ATP+GTP)/(UTP+CTP) ratio, and an increase of FBP level, glutamine consumption and cell proliferation (30). In addition, it has been reported that a low glycolytic NIH3T3 cell line is characterized by high pyruvate and glutamine consumption and a large number of dimeric forms of PKM2, which is consistent with high FBP levels, low (ATP+GTP)/(CTP+UTP) ratios and high diffusivity (20). This interaction is very important for the transformation potential of tumor cells (94).

PKM2 not only plays a glycolytic role in the cell but also acts as a protein kinase. Yang et al (95) found that the activation of EGFR induced PKM2 translocation to the nucleus, and the interaction of PKM2 with β-catenin led to the inhibition of histone deacetylase 3 and an increase in cyclin D1 expression from the promoter, thus promoting tumor cell proliferation.

Signal transducer and activator of transcription 3 (STAT3) is also a substrate of PKM2 kinase activity. Nuclear PKM2 phosphorylates STAT3 at Y705 and then activates MEK5 transcription (96). In addition, CD276 induces PKM2 phosphorylation through the STAT3 signal pathway, thus promoting glucose metabolism in tumors (97). In lung cancer cells, eukaryotic EF2K forms a complex with PKM2 and STAT3, and phosphorylates PKM2 at T129, resulting in a decrease in PKM2 dimerization. Subsequently, PKM2 blocks STAT3 phosphorylation and STAT3-dependent c-Myc expression (98). A study on HPV16-positive cervical cancer cell lines (SiHa and CaSki) and primary tumor tissues showed that STAT3 plays a role in the occurrence and development of cervical cancer (99). The activity of STAT3 was positively correlated with the expression of HPV16 E6 and E7, and negatively correlated with the expression of p53 and pRB (100). E6 is mainly responsible for the phosphorylation of STAT3 in HPV keratinocytes, while E5, E6 and E7 can induce STAT3 tyrosine phosphorylation in HPV cervical cancer cells (101). However, the direct dependence of STAT3 on the activation of PKM2 to induce the formation of cervical cancer cells has not yet been studied.

PKM2 can interact with the transcription factor encoded by the octamer-binding transcription factor-4 (Oct-4) gene, which plays an important role in maintaining the pluripotent state of embryonic stem cells (102). The pituitary-specific Pit-1 (POU) DNA binding domain of Oct-4 is necessary for interaction with PKM2, which positively regulates the transactivation potential of Oct-4. In addition, ectopic expression of PKM2 enhances Oct-4-mediated transcription (103). A study demonstrated that HPV E7 also specifically binds to the Oct-4 POU domain, and the expression of E7 in differentiated cells can stimulate the transactivation of Oct-4-mediated distal binding sites (104). However, whether the combination of E7 and Oct-4 has an impact on PKM2 also needs further study (Fig. 1).

7. E7, PKM2 and the tumor immune microenvironment (TIME)

The TIME has attracted increasing attention in recent years, especially in clinics (105). With the progress of technology, the understanding of the complexity and diversity of the TIME and its impact on treatment response has deepened. The TIME is composed of factors such as blood vessels, myeloid-derived suppressor cells, antigen-presenting cells, lymphocytes, dendritic cells, fibroblasts, extracellular matrix, cytokines and growth factors (106). These components can functionally sculpt the TIME by secreting various cytokines, chemokines and other factors, resulting in the anticaner immune response, and can have an important influence on the occurrence and development of cancer. Previous studies have shown that PKM2 plays an important role in the TIME by influencing the Warburg effect of cancer cells, immune cells (such as lymphocytes, dendritic cells and macrophages) and immune checkpoints [such as programmed cell death ligand-1 (PDL1)] (34,107). However, it is not clear whether PKM2 plays a vital role in cancer metabolism and immunity.

In the process of tumorigenesis, lymphocytes are activated and proliferate, which is characterized by a significant increase in aerobic glycolysis (108). Meanwhile, T-cell proliferation increases the expression of PKM2 (especially in CD4+ T cells), and the accumulation of PKM2 in the nucleus affects the metabolic reprogramming of CD4+ T cells in turn (109). A previous
study has shown that PKM2 controls T-cell activation induced by homocysteine (8). In addition, TEPP-46 and DASA-58, (two common activators of PKM2) can convert PKM2 dimer into tetramer and inhibit PKM2 nuclear transfer (110,111). TEPP-46 can also restrict the development of Th17 and Th1 cells in vitro and inhibits T cell-mediated inflammation (112).

Nuclear localization of PKM2 is important for the regulation of the TIME, and the role of HPV E7 is essential in this process. PKM2 has been found to play a role in the differentiation and function of immune cells (113,114). PKM2 can form transcriptional complexes with HIF-1α, and then regulate the release of high mobility group box-1 from activated macrophages. The latter can release inflammatory factors to change the immune microenvironment (115). In addition, nuclear translocation of dimer PKM2 leads to phosphorylation of STAT3 in lipopolysaccharide (LPS)-stimulated coronary artery disease macrophages and promotes IL-1β and IL-6 transcription (116). Moreover, the transformation of PKM2 from a dimeric to a tetrameric conformation effectively inhibits LPS-induced nuclear translocation and subsequent expression of IL-1β and a series of other HIF-1α-dependent genes, which is important for the Warburg effect in macrophages (Fig. 2) (117).

It is worth noting that PKM2 also plays a key role in the differentiation of different immune cells; for example, PKM2 participates in T-cell differentiation by regulating STAT1/STAT4 and then inducing type 1 T helper (Th1) cell formation (118). In addition, PKM2 interacts with STAT3 to enhance its activation after migration to the nucleus, thus increasing the differentiation of Th17 cells (113). Furthermore, IL-23 is an important cytokine in the polarization of Th17, which can induce the phosphorylation of PKM2 and STAT3 in T cells and promote their nuclear translocation (119). Moreover, previous studies have indicated that nuclear PKM2 can also stimulate the proliferation of cancer cells by regulating the activity of STAT1 and STAT5 (Fig. 2) (120,121).

Finally, as one of the most promising immunotherapy methods, the use of immune checkpoint inhibitors, especially PDL1, has attracted extensive attention in recent years. Immune checkpoint inhibitors have been proven to have strong immunomodulatory effects through their function as negative regulatory factors of T cells (122). PDL1 can be expressed in a variety of cell types, including cancer cells, and PKM2 is crucial for PDL1 expression according to the report by Luo et al (58). PKM2 and HIF-1α can bind to two hypoxia-response elements (HRE1 or HRE4) of the PDL1 promoter at the same time to promote the transcription of PDL1 (123). In cervical cancer, the high expression of E7 can promote the nuclear localization and phosphorylation of PKM2 thus promoting the proliferation of cancer cells, and increase the activity of HIF-1α and then interact with PKM2 to enhance immune surveillance of cancer escape (Fig. 2).

8. Conclusion

During energy metabolism, cervical cancer cells are inclined to utilize glycolysis under aerobic conditions. As a rate-limiting enzyme in glycolysis, PKM2 plays an important role in the aerobic glycolysis pathway. Although HPV E7 can also promote the Warburg effect, the mechanism is not clear. A large number of studies have shown that there is some regulatory relationship between E7 and PKM2, but the specific mechanism is still mostly unknown and needs further study. Since the intracellular mechanisms influenced by HPV E7 interaction with PKM2 are much more complex than previously assumed, insights gained from the present review may be helpful for further research and clinical treatment of cervical cancer in the future.
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Availability of data and materials

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Authors' contributions

CG was the major contributor to writing the manuscript. MJ, YS and JW revised the manuscript. YZ made substantial contributions to conception and design and gave the final approval of the version to be published. MJ involved in revising the manuscript critically for important intellectual content. All authors read and approved the final manuscript. MJ involved in initial contributions to conception and design and gave the final approval of the version to be published. YZ, CG and FD contributed equally to this study.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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