Introduction to DOK2 and its potential role in cancer

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Abstract

Cancer is a complex, multifactorial disease that modern medicine ultimately aims to overcome. DOK2 is a well-known tumor suppressor gene, and a member of the downstream protein Dok family of tyrosine kinases. Through a search of original literature indexed in Pubmed and other databases, the present review aims to extricate the mechanisms by which DOK2 acts on cancer, thereby identifying more reliable and effective therapeutic targets to promote enhanced methods of cancer prevention and treatment. The review focuses on the role of DOK2 in multiple tumor types in the lungs, intestines, liver, and breast. Additionally, we discuss the potential mechanisms of action of DOK2 and the downstream consequences via the Ras/MPAK/ERK or PI3K/AKT/mTOR signaling pathways.

Keywords

tumor; DOK2; tyrosine phosphorylation; EGFR; SHIP-1; DOK family

1. Introduction to DOK2

There are seven members of the DOK family of proteins downstream of tyrosine kinase, namely DOK1-7. DOK1 and DOK2 are able to recruit p120rasGAP, thereby inhibiting the Ras-MAPK-ERK pathway, playing important roles in cell growth and development. DOK3 can negatively regulate activation of c-Jun N-terminal kinase (JNK) and Ca^{2+} mobilization. In addition, DOK3 also binds the structural domains, inositol-5-phosphatase (SHIP-1) and growth factor receptor-bound protein2 (Grb2), which are involved in cellular regulation. Although DOK4, DOK5, and DOK6 do not modify the Ras pathway, they are expressed in a variety of nerve tissues, together with Glial cell-derived neurotrophic factor receptor C (c-Ret), playing an important role in the growth and development of nerve cells. Additionally, DOK7 has been shown to be important in the formation of neuromuscular synapses (Grimm *et al*.2001; Mashima *et al*.2009; Ueta *et al*.2017). The principal focus of the present manuscript is DOK2, also known as DOKR and FRIP, a member of the DOK family located on human chromosome 8p21.3 and a well-known tumor suppressor gene. As a member of the joint protein tyrosine kinase family, DOK2 acts through tyrosine kinase epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and human epidermal growth factor receptor (Her-2/NEU-8) via negative feedback modulation of the signal transduction of protein tyrosine kinase (PTK). In addition, DOK2 can also suppress the activity of receptor tyrosine kinases. Src family kinases recruit C-Src tyrosine kinase (Csk), which inhibits mitogen-activated protein kinase (MAPK) and Akt (also known as Protein Kinase B) phosphorylation, which influence DOK2 and which can be stimulated by EGF (Mashima *et al*.2013; Jones *et al*.1999; Suzu *et al*.2000; Berger *et al*.2013; Van *et al*.2005). The DOK family has a common topological structure, namely a homologous domain (PH) at the N-terminal, a central tyrosine phosphorylation binding domain (PTB), and c-terminal SH2 and SH3 target sequences (PRR). The PH sequence is mainly involved with tyrosine phosphorylation and cell membrane localization of proteins (Suzu *et al*.2000). The PTB is not only associated with tyrosine phosphorylation but also binds to the PTB sequence on EGFR, resulting in a series of cascade reactions (Jones *et al*.1999). The PTB domain is an intracytoplasmic portion of the DOK protein. PTB contains NPXY and NXXY

motifs through which it can bind to cell surface receptors (Smith *et al*.2006). SH2 and SH3 domains at the C-terminal have seven PXXP motifs containing not only tyrosine phosphorylation residues and proline, but also RasGAP, Nck, Csk, and SHIP-1 sites, producing a cascade reaction (Di *et al*.1998). When undergoing stimulation by a growth factor, DOK proteins become localized to the membrane signaling complex under the influence of PH and PTB domains, with increasing numbers of proteins that are recruited to participate in cascade reactions under the action of the PXXP motif and tyrosine phosphorylation residues on SH2 and SH3 (Berger *et al*.2013). The present study will focus on the relationship between DOK2 and cancer, and so introduce the functions of DOK2, as displayed in Figure 1.

2. DOK2 and physiological cellular function

2.1. DOK2 and cell fusion and proliferation

Shuhei Kajikawa et al. demonstrated that DOK2 is expressed in both macrophages and monocytes in blood, with DOK2 regulating multiple signaling pathways via negative feedback which affects osteoclast proliferation. In a mouse model in which the DOK1 and DOK2 genes had been knocked out, animals were found to have larger numbers of osteoclasts and were more likely to develop symptoms of bone deficiency than wild-type mice (Kajikawa *et al*.2018). In addition to bone, the downregulation or deletion of DOK l and DOK2 from blood has been shown to increase the incidence of chronic myelogenous leukemia and myeloproliferative diseases. The deletion or low expression of the DOK2 gene promotes the fusion and proliferation of a number of cell types. DOK1 and DOK2 regulate the development and function of natural killer (NK) cells, which play an important role in the immune response and tumor inhibition. Overexpression of DOK proteins in human NK cells can stimulate the activation of receptors on NK cells and thus inhibit their activity. In mice, the deletion of DOK1 and DOK2 genes has been shown to cause maturation defects in NK cells and increase the release of the cytokine interferon gamma (IFN-γ) (Celis-Gutierrez *et al*.2014). Furthermore, cell proliferation and differentiation are regulated by cytokines that bind to their corresponding receptors, which activate a variety of tyrosine kinases,

including JAK kinases and Src family kinases. In turn, cytokines can also stimulate DOK2 phosphorylation. Studies have demonstrated that the expression of DOK2 in M-NFS-60 cells causes the release of a large number of cytokines that inhibit cellular proliferation and differentiation (Suzu *et al*.2000).

2.2. DOK2 and regulation of the cell cycle of hematopoietic stem and progenitor cells

Emile Coppin et al. demonstrated that DOK1 and DOK2 play significant roles in the proliferation of myeloid cells (Coppin *et al*.2016). In elderly mice, the downregulation or deletion of DOK1 and DOK2 induces myeloproliferative disease. Greater graft survival and transient medullary amplification capability have been observed in hematopoietic stem cells after the knockout of DOK1 and DOK2 genes. In hematopoietic progenitor cells, the expression of DOK l and DOK2 inhibits the cell cycling of hematopoietic progenitor cells, thus affecting their proliferation. Therefore, DOK1 and DOK2 can regulate the signaling pathways of hematopoietic cell growth and differentiation and therefore, they affect the growth of myeloid hematopoietic cells.

2.3. DOK2 and platelet regulation

Hughan et al. confirmed that DOK2 plays an important role in integrin-induced signal transduction from extracellular stimuli to intracellular signaling (Hughan *et al*.2000). In addition, immune complexes formed by DOK2 and integrin αIIβ3 are also expressed downstream of Src family kinases. DOK2 plays an important role in regulating the physiological function of platelets since thrombin and αIIβ3 receptors on platelets can stimulate the phosphorylation of platelet tyrosine residues. It has been shown that DOK2 is regulated in platelet-derived microparticles (PMPs) following stimulation by other platelets (Bidkhori *et al*.2013). In addition, DOK2, a platelet-specific collagen receptor glycoprotein V1 (GPVI) signaling protein, has been found to undergo a higher level of tyrosine phosphorylation in response to the GPV1-specific agonist CRP (collagen-related peptide), a significant factor at sites of coronary artery occlusion (Vélez *et al*.2016).

2.4. DOK2 is a negative regulator of T-cell receptor signaling

It has been proposed by Yasuda et al. that DOK2 functions as a negative regulator of

T-cell receptor signaling. The interaction between a TCR complex and an antigenic peptide is a central event in the immune response. It has been observed that the response in mice to thymo-dependent antigens and the response of T-cells to TCR stimulation is enhanced in the absence of DOK1 and DOK2, principally manifested as the tyrosine phosphorylation activation of ZAP-70, the proliferation of T-cells, and production of cytokines (Yasuda *et al*.2007). DOK proteins play key roles in establishing a T-cell signal negative feedback loop. These structure-related adaptor molecules contain a Pleckstrin homology (PH) domain, usually a lipid/protein interaction module. Experiments conducted by Guittard et al. demonstrated that the PH domain is required for DOK phosphorylation which triggered DOK1 and DOK2 inhibition in activated T-cells, while it has been found that the tyrosine phosphorylation of DOK1 and DOK2 proteins can be induced by inositol 5-phosphatase containing SH2 domains produced by T-cells, triggered by TCR (Guittard *et al*.2009). DOK1 and DOK2 play key roles in the linker for activation of T-cells (LAT)-dependent negative feedback loop, which attenuates early TCR signaling. However, TCR can induce polymolecular complexes, including DOK2, SHI-1, and GRB-2, which assemble into LAT. LAT and SHI-1 also mediate tyrosine phosphorylation in DOK2. When DOK1 and DOK2 are inhibited, TCR-mediated IL-2 production and signaling transduction have been found to be enhanced (Dong *et al*.2006). CD4 can enhance the sensitivity of the T-cell interaction with antigen peptide/MHC II molecular complexes. Waterman et al. demonstrated that CD4 aggregation leads to LCk-dependent phosphorylation of the RasGAP adaptor downstream of DOK-1/2 and inositol 5-phosphatase-1 (SHIP-1), leading to the binding of these two molecules. They hypothesized that the loss of function in $CD4^+$ T-cells in HIV-1 may be due to the aggregation of gp120 with CD4 and subsequent inhibition of TCR activation in SHIP-1 and DOK1/2 pathways. It has been established that the SHIP pathway is necessary for CD4-mediated inhibition of calcium mobilization and proliferation of TCR stimulation activation (Waterman *et al*.2012). Retrovirus-mediated expression of DOK2 kinase-related protein, also known as DOK-R, in bone marrow cells has been shown by Gugasyan et al. to significantly

inhibit the cytokine-dependent colony formation of hematopoietic progenitor cells, reduce the proliferation of thymus cells, and selectively inhibit the development of T-lymphocytes (Gugasyan *et al*.2002).

2.5. DOK2 and tyrosine channels

DOK2, a downstream product of tyrosine kinase negatively regulates the signaling pathway of tyrosine kinase. DOK2 inhibits MAPK and Akt signaling, thus inhibiting cell proliferation and migration via two forms of inhibitory mechanism. It has been shown that DOK2 recruitment of the negative regulator of Ras signaling in the Ras-Raf-MAPK pathway inhibits MAPK activity, while DOK2 inhibits Akt phosphorylation through the phospholipinositol 3-carboxykinase (PI3K)-Akt pathway (Niki *et al*.2004). In T-cells, for example, it has been found that DOK2 inhibits tyrosine phosphorylation and the Ras signaling pathway, thereby participating in the negative feedback regulation of Tec (Gérard *et al*.2004). In human bone marrow cells, CD200R directly recruits DOK2 and activates RasGAP, thereby inhibiting bone marrow cells to become activated (Mihrshahi *et al*.2009). Toll-like receptor II (TLR-2) strengthens DOK2 phosphorylation, with negative feedback modulating Ras-ERK activation (Downer *et al*.2013). After stimulation by EGF, DOK2 becomes phosphorylated, directly combining with EGFR tyrosine phosphate, then with Src family kinase (SFK) under the action of Csk, which inhibits MAPK, ERK, and Ras pathways, exacerbated by EGF (Berger *et al*.2013; Van *et al*.2005;Asati *et al*.2016). The combination of platelet-derived growth factor (PDGF) and PDGFR facilitates the phosphorylation of DOK2, thereby inhibiting the PI3K-Akt signaling pathway. Like DOK2, insulin, and insulin-like growth factor expression suppress the PI3K-Akt-mTOR and Ras-MAPK pathways (Solarek *et al*.2019). Early research demonstrated that Lck tyrosine kinase participates in signal transduction through T-cell surface receptors, such as TCR/CD3, CD2, and CD28. Ne'Morin et al. found that the cell surface receptor CD2 mediates Lck activation leading to specific phosphorylation of RasGAP, P56DOK (DOK2), and P62DOK (DOK1) (Némorin *et al*.2000). In addition, they also demonstrated that phosphorylation of Ras GAP, DOK2, and DOK1 is associated with increased intracellular Ca^{2+} concentration and that the

association between Lck and DOK1 is formed through an SH2-mediated interaction. They also found that the phosphorylated tyrosine (PTB) binding domain of DOK2 and DOK1 mediates their isotypic and heterologous interactions. PTB-mediated oligomerization of Dok-1 and Dok-2 has been shown to be a requirement for DOK tyrosine phosphorylation and functionality (Boulay *et al*.2005).

2.6. DOK2 is a negative regulator of lipopolysaccharide-induced signaling

Endotoxin is a form of bacterial lipopolysaccharide (LPS). Shinohara et al. demonstrated that DOK1 and DOK2 are negative regulators of tumor necrosis factor α (TNF-α) and nitric oxide (NO) produced after treatment of macrophages with LPS. DOK1 and DOK2 are known to be important adapters of the negative regulation of ERK. Shinohara verified that forced expression of DOK1 and DOK2 inhibits LPS-induced ERK activation and TNF-α production. It was also found that mice lacking DOK1 and DOK2 are allergic to LPS (Guittard *et al*.2009).

2.7. DOK2 and type I Fc receptors

The expression of type I Fc receptors (FcIR) on mast cells causes activation of a biochemical cascade reaction, leading eventually to the release of inflammatory cytokines and a change in cell morphology and adhesion. It has been found that DOK1 and DOK2 are involved in an FcIR cascade in mucosal-type cells in RBL-2H3 rats. FcIR has also been shown to stimulate the phosphorylation of DOK1 and DOK2 through tyrosine (Abramson *et al*.2007). In addition, DOK1 and DOK2 may also be key to cell cytoskeletal rearrangements following FcIR stimulation.

2.8. DOK2 regulates memory CD8+ T-cells

The strength and nature of TCR signaling affect the differentiation and function of effector and memory CD8+ T-cells. DOK1 and DOK2 are expressed on T-cells and negatively regulate the TCR signaling pathway *in vitro*. Laroche et al. investigated the role of DOKl and DOK2 proteins in the regulatory response of CD8+ T-cells to vaccinia viral infection (Laroche *et al*.2016). The response of wild-type cells to cowpox virus expressing the OVA peptide SIIFEKL was compared to that of CD84 OT-1 cells in which both DOK1 and DOK2 were silenced. They found that CD8- T-cell proliferation was inhibited because the T-cells displayed serious survival

defects. In addition, CD8+ T-cells expressing DOK1 and DOK2 were also found to express TCR on their cell surface following stimulation by viral antigens *in vivo*, thus promoting the expression of granzyme B and TNF *in vitro*. This indicates that DOK1 and DOK2 negatively regulate the overactivation of CD8+ T-cells and promote the formation of memory cells.

2.9. DOK2 with CD200 and CD200R

It has been shown that the interaction between membrane glycoprotein CD200 and its homologous receptor CD200R plays an important role in the static maintenance of microglia (Lyons *et al*.2012). DOK2 phosphorylation is a key event that mediates the role of CD200 fusion protein (CD200FC) because DOK2 deletion blocks the activation of CD200FC on microglia cells and the production of cellular inflammatory cytokines. CD200R is located in the cytoplasm and has 67 amino acids, containing 3 tyrosine residues and an NPXY motif. The interaction between CD200 and CD200R induces phosphorylation of these residues, initiating a signaling cascade and recruitment of DOK1 and DOK2 proteins, which activate RasGAP and SHIP, SH2-containing proteins. CD200R and DOK2 can stimulate microglia proliferation, and so CD200R and DOK2 may play roles in neurodegenerative diseases. The CD200/CD200R pathway is also of great significance for the treatment of leukemias. Viruses can mimic host proteins that produce CD200 and suppress the host antiviral response. Therefore, CD200 is also a marker of human cancer or cancer stem cells. Viruses suppress immune recognition, leading to immune escape by inhibition of activated white blood cells bearing CD200R (Mihrshahi *et al*.2010). In human medullary cells, CD200R is able to recruit DOK1 and DOK2, thereby inhibiting the Ras pathway. Furthermore, it has also been found that DOK2 can directly interact with the NPXY motif in human CD200R, leading to the activation of RasGAP, thereby inhibiting the activation of human medullary cells (Rijkers *et al*.2007). In mouse medullary mast cells, CD200R is phosphorylated on tyrosine residues following the binding with its ligand, followed by binding to DOK1 and DOK2. It has been found that DOK1 binds to SHIP, and both DOK1 and DOK2 recruit RasGAP, leading to the inhibition of the Ras-MAPK signaling pathway (Zhang *et al*.2004; Zhang

et al.2006). Studies have also shown that CD200R1 may also be associated with head and neck squamous cell carcinoma due to the action of DOK2, as shown in Table 1 (Chang *et al*.2020).

3. DOK2 and pathology

3.1. DOK2 and herpes simplex virus 1

After herpes simplex virus-1 (HSV-1) infection, viral envelope protein vp11 / 12 can bind to DOK2 through its SHC-binding motif, inducing tyrosine phosphorylation and selective degradation of DOK2. Lahmidi *et al.* speculated that this may be an immune escape mechanism through inactivation of T-cells or inhibition of T-cell immune function (Lahmidi *et al*.2017). When HSV-1 becomes a latent infection, DOK2 can promote the survival of HSV-1-specific CD8+ T-cells in lymphoid tissues, such as the spleen and draining lymph nodes, and also non-lymphoid tissues, such as the cornea and trigeminal ganglion (TG). Its deficiency has been shown to promote activation of latent HSV-1 *in vitro* (Lahmidi *et al*.2017).

3.2. DOK2 and glial inflammation

Toll-like receptor 2 (TLR-2) is a bridge between specific and nonspecific immunity, through recognition of pathogen-related molecular patterns, triggering signal transduction and leading to the release of inflammatory mediators. Studies by Downer and others have shown that TLR-2 enhances tyrosine phosphorylation of DOK1 and DOK2 in astrocytes and microglia. In astrocytes transfected with DOK1 and DOK2 siRNA, the production of NF-κB and IL-6 induced by TLR-2 was found to be enhanced, indicating that DOK1 and DOK2 proteins affect the release of inflammatory mediators induced by TLR-2 through negative feedback regulation (Solarek *et al*.2019).

3.3. DOK2 and leishmaniasis infection

Previous studies have shown that DOK1, DOK2, and DOK3 are targets of GP63, a metalloprotein associated with Leishmania. Álvarez de Celis et al. found that tumor necrosis factor and nitric oxide expression declined in macrophages lacking Dok1 and Dok2 expression treated with Δgp63 protein or wild type L. major promastigotes

compared with wild-type macrophages, indicating that DOK proteins may be an important regulatory factor in macrophages infected with Leishmania (Álvarez *et al*.2015).

3.4. DOK2 and peritoneal fibrosis

Peritoneal fibrosis (PF) is a recognized complication associated with continuous peritoneal dialysis, characterized by early reversible epithelial to mesenchymal transition (EMT) (Zhang *et al*.2019). The long noncoding RNA AK089579 inhibits EMT in peritoneal stromal cells (PMC) by the competitive binding of microRNA-296-3p to DOK2. As is well known, DOK2 is a target gene of miRNA296, so AK089579 can regulate the expression of DOK2 by regulation of miRNA296 through the JAK2-STAT3 signaling pathway. AK089579 can inhibit the activation of the JAK2-STAT3 signaling pathway, and indirectly upregulate the expression of DOK2 by combining with miRNA296, thus inhibiting migration, invasion, and EMT in the PMC in a murine model of PF. In addition, upregulation of miRNA296 and down-regulation of DOK2 were found to display opposite effects. In conclusion, DOK2 may play a key role in PF, as shown in Table 2.

4. DOK2 and tumors

4.1. DOK2 and colorectal cancer

Colorectal cancer is the most common malignant tumor of the digestive system. Its morbidity and mortality are on the rise, currently ranking third among malignant tumors. Although many forms of treatment can improve the postoperative survival of patients with colorectal cancer, biomarkers for early diagnosis and poor prognosis of colorectal cancer remain lacking. Thus, it remains an important goal to identify new biomarkers for the disease. Research by Xianmei Wen et al. indicates that of 102 patients with postoperative colorectal cancer, 33.3% did not express DOK2, a subgroup that generally displayed poor prognosis, with a five-year survival rate of only 59.1%. In comparison, the five-year survival rate of patients expressing DOK2 was as high as 76.4% (P=0.0328). The expression of DOK2 was found to be low in 34 patients with poorly differentiated colorectal cancer. Compared with colon cancer

tissue which is generally observed to have high DOK2 expression, highly differentiated and chronically differentiated adenomas lacked the expression of DOK2 (Wen *et al*.2015). These observations suggest that low DOK2 expression may be a biomarker of poor prognosis in patients undergoing colorectal cancer resection. Moreover, DOK2 may also play an important role in its early diagnosis and treatment. However, at present, the consequences of low expression of DOK2 are not been fully understood, and additional studies are required to confirm the mechanism of DOK2 in the treatment of colorectal cancer and other cancers.

4.2. DOK2 and leukemia

The combined loss of DOK1 and DOK2 can trigger chronic myeloid leukemia (CML)-like myeloproliferative disease (MPD) when completely explicit. Their lack results in the proliferation or survival of hematopoietic cells in the presence or absence of growth factors, respectively. This indicates that both DOK1 and DOK2 play key synergistic roles in the homeostasis of hematopoiesis and inhibition of tumors, allowing resistance to p210 BCR-ABL-driven leukemia and lymph node formation (Niki *et al*.2004). In a study published by Yasuda et al., it was revealed that DOK1 and DOK2 play synergistic roles in the negative regulation of a number of cytokines, except G-CSF (Yasuda *et al*.2004). They also synergistically inhibit extracellular-regulated protein kinase (ERK) and protein kinase B (PKB) when stimulated synergistically by cytokines. In addition, DOK1 and DOK2 inhibit cytokine-mediated proliferation and anti-apoptotic signals in myeloid cells. In a study published by Niki et al., DOK1 and DOK2 were found to inhibit the development of myeloid leukemia and CML-like disease mutations in mice (Niki *et al*.2004; Yasuda *et al*.2004)). DOK1 and DOK2 were also shown to prevent HL-60 cells from losing viability following long-term exposure to high serum levels. When induced by trans-retinoic acid (RA) and 1,25-dihydroxyvitamin D3 (VD3), ectopic expression of DOK2 was shown to increase in HL-60 cells following the arrest of growth, differentiation, and G0/G1 cell cycle arrest induced by RA and VD3, resulting in increased phosphorylation of extracellular regulated protein kinase (ERK1/2)(Lamkin *et al*.2006).

Dasatinib has been found to inhibit the function of c-Abl by reducing the phosphorylation of DOK2, a downstream target of c-Abl, during the treatment of primary chronic lymphoblastic leukemia (CLL), inducing H2AX phosphorylation, causing CLL lymphocytes to be sensitive to chlorambucil and fludarabin (Amrein *et al*.2008). In chronic myeloid leukemia (CMML), DOK2 undergoes point mutation, affecting the DOK2 PTB domain. For example, the L238P mutation can modify the PTB domain, preventing binding to the phosphotyrosine peptide, and the tyrosine-phosphorylated DOK1 protein. It also leads to the loss of DOK2 functionality and the failure to inhibit ERK activation (Coppin *et al*.2015). DOK2 and SHIP1 proteins are the most significant constitutive phosphorylation substrates of BCR-ABL kinase. In a study by Xiquan Liang et al., DOK2 was found to be phosphorylated at tyrosine 299, while imatinib was shown to inhibit the tyrosine phosphorylation of DOK2 (Liang *et al*.2006). In two groups of experiments by Takeo Ohsugi et al., it was first shown that, compared with uninfected T-cells, DOK2 expression is significantly reduced in transformed T-cells (MT-2 and hut-102) infected with HTLV-1, and TL-Om1 cells from adult T-cell leukemia/lymphoma (ATLL) patients (Ohsugi *et al*.2016). They subsequently studied human T-cell leukemia virus type 1 transferred into mice. The HTLV-1 transgenic (TG) mice expressed DOK2 in mature thymocytes and peripheral lymphocytes. We have observed that DOK2 expression in TG mice is significantly lower than in non-TG mice prior to exhibiting disease symptoms, with the downregulation of DOK2 expression the first step in the development of disease characterized by low DOK2 gene expression (Ohsugi *et al*.2017). Knockout of DOK2 mRNA in mouse erythrocytic leukemia cells with high DOK2 expression has been found to increase the expression of Kruppel like factor (KIf1). Experiments by Tanaka demonstrate that DOK2 binds directly to the KIf1 promoter, controlling KIf1 expression through transcriptional regulation (Tanaka *et al*.2014). Pin Fang He et al. have also investigated the decreased expression of DOK1 and DOK2, finding an association with hypermethylation of the promoters, allowing prediction of adverse prognosis of acute myeloid leukemia (AML) (He *et al*.2018).

4.3. DOK2 and invasive histiosarcoma

Mashima et al. obtained TKO mice by knockout of the DOK1/2/3 genes. The results demonstrate that invasive tissue sarcoma (HS) is significantly associated with a lethal phenotype in TKO mice, finding that the HS observed in the mice was highly invasive and transplantable. Abnormal aggregation of pulmonary macrophages in TKO mice was also observed, indicating that DOK1/2/3 are negative regulators of the macrophage response to macrophage colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) (Mashima *et al*.2010).

4.4. DOK2 and lung cancer

In a study by Chen et al., DOK2 and Dusp4 were found to display both tumor suppressive and MAPK inhibitory functionality due to haploid deficiency. The complex heterozygosity of DOK2 and Dusp4 enhance sensitivity to MEK inhibition, and both inhibit MAPK activation and cell proliferation. It was found that DOK2 and Dusp4 heterozygosity synergistically promotes the occurrence of lung tumors (Chen *et al*.2019). It is well known that DOK2 is a human lung tumor suppressor, and Berger et al. further confirmed that DOK2 is a target for genomic deletion and down-regulation of human lung cancer, with DOK2 able to inhibit the proliferation of lung cancer both *in vitro* and *in vivo*. They also concluded that single or compound knockout of DOK1/2/3 can cause lung cancer in mice and that the rate of incidence of lung adenocarcinoma increases with the number of missing alleles. It has been found that DOK2 expression in lung cancer cell lines is very low or undetectable (Berger *et al*.2010). Berger has also demonstrated that DOK2 inhibits EGF-induced Ras and ERK activity, interacting with EGFR. In other words, the induction of EGFR activation by ligands or carcinogenic mutations induce DOK2 to recruit the EGF receptor complex and rasa1. In addition, DOK2 has been shown to inhibit the expansion of EGFR mutant lung adenocarcinoma cells (Berger *et al*.2013).

4.5. DOK2 and renal cancer

Globally, renal cancer is the 16th most common disease, with 403,262 new cases and 175,098 deaths in 2018. Solarek et al. compared HEK293 renal cancer cells with PCS-400-010 normal renal cells and evaluated cell growth, viability, and mobility following hormone stimulation, also measuring insulin and insulin-like growth factor

1 receptor (IGF-1) expression. The results indicate that insulin and IGF-1 promote the growth and migration of renal carcinoma cells (Solarek *et al*.2019; Kužma *et al*.2019). In addition, the expression of insulin and IGF-1 promote the expression of genes inhibitory to the PI3K-Akt-MTOR and RAS-MAPK signaling pathways, similar to the functions of genes such as DOK1, DOK2, INS, and FRS3. In addition, these genes also encode insulin receptor-related proteins, although they are not expressed in renal cancer cells. Therefore, this suggests that low DOK2 expression causes low insulin and IGF-1 expression, and so the rapid growth and migration of renal cancer.

4.6. DOK2 and breast cancer

Breast cancer is among the two most deadly cancers in women in Asia, with a very high incidence. Although the survival rate of patients with breast cancer is as high as 65% with advanced treatment, the identification of novel biomarkers for its early diagnosis and poor prognosis remains a challenge(Jambor *et al*.2019). Huang et al. demonstrated that low expression of DOK2 and RASA1 is related to poor differentiation in breast cancer, and the deletion of DOK2 and RASA1 is related to increased tumor size, increased proportion of axillary lymph node metastasis, and higher clinical stage. The absence of DOK2 and RASA1 may cause activation of Ras extracellular signaling that regulates the kinase cascade, leading to cell cycle abnormalities that affect tumor size and metastasis (Huang *et al*.2017; Ghanem *et al*.2014; Zhang *et al*.2020). Ghanerm et al. found that decreased DOK2 mRNA expression in human breast cancer cells leads to higher TNM staging, while patients with high expression of DOK2 have a lower risk of recurrence and distant metastasis following surgical resection (Ghanem *et al*.2014). When treating breast cancer, the lack of the DOK2 gene reduces the level of apoptosis leading to drug resistance. In addition, DOK2 promotes the recycling of EGFR by the C-Src inhibitor Csk that inhibits the activation of MAPK, which is closely related to the occurrence and development of breast cancer (Van *et al*.2005). In summary, the deletion or low expression of DOK2 may represent low differentiation in breast cancer cells and related to poor prognosis in patients. Therefore, DOK2 may play an important role in the early diagnosis and treatment of breast cancer.

4.7. DOK2 and glioma

Glioma is the most common primary intracranial tumor, accounting for 27% of all tumors of the central nervous system(Herbet *et al*.2018). Pramod et al. confirmed that NC, a well-known anticancer drug, can recruit DOK2 and Caspase3 that affects the death of glioma in C6 mice and U87 human malignant glioma cells. Therefore, the overexpression of DOK2 protein may be related to poor prognosis in human glioma (Deshpande *et al*.2018).

4.8. DOK2 and lymphoma

Lymphoma is not uncommon in China, with an incidence of 4-5 per 100,000 population, and so it is one of the ten most common malignant tumors. In patients with lymphomas, the disease is often caused by the multiple cloning of a cell with a particular TCR rearrangement, while it is also partly caused by loss of TCR expression. Therefore, we speculated that the TCR pathway may play an important role in the occurrence and development of T-cell lymphoma. Miyata-Takata et al. identified components of the TCR pathway in 91 formalin-fixed paraffin-embedded lymphatic tissues using immunohistochemical techniques. The results indicate that ZAP70 is expressed in 94% (83/88) of cases, GRAP2/GADS in 68% (60/68), DOK2 in 42% (38/90), LCK in 35% (31188), and ITK in 10% (9/90). Thus, iDOK2 appears to affect the TCR expression pathway and regulates the growth of T-cell lymphomas (Miyata *et al*.2018). Furthermore, immunostaining of proteins suggests that DOK2 expression is also lower in both normal T-cells and those of lymphoma patients. It thus plays an important role in the differentiation of cells. Therefore, there may be a significant role for DOK2 in the treatment of T-cell lymphoma.

4.9. DOK2 and astrocytoma

Astrocytoma is a common malignant tumor of the brain. Although clinical research has made good progress, the median survival of patients with malignant astrocytoma remains very low. From 47 tissue samples of patients undergoing resection of astrocytoma, PCR and Western blot analysis has found that DOK2 protein is overexpressed in 83% of stage GIII and GIV astrocytoma, and in 30% of stage GII tissues. In a follow-up investigation, the median survival of patients in the

DOK2-overexpression group was only 20 months (95% CI: 0.083 - 0.49) (Deshpande *et al*.2018). In conclusion, DOK2 overexpression is associated with poor prognosis in astrocytoma and may represent an effective target for its treatment.

4.10. DOK2 and other tumors

It has been shown that inhibition of DOK2 increases platinum resistance in ovarian cancer cells. In addition, loss of DOK2 protects against apoptosis and anoikis, that is, loss of DOK2 can induce resistance to carboplatin in ovarian cancer cells (Fang *et al*.2018). Concversely, inhibition of DNA methylation has been found to leads to the upregulation of DOK2 expression, causing ovarian cancer cells to be sensitive to platinum-based drugs (Lum *et al*.2013), and should be considered when Appraising the prognosis of ovarian cancer patients. Furthermore, the low frequency of missense mutants of DOK2 is significantly correlated with the increased incidence of pancreatic adenocarcinoma (PDAC) (Chang *et al*.2013). Additionally, it can also be used as a marker of poor prognosis after radical mastectomy (Miyagaki *et al*.2012), as shown in Table 3.

5.Mechanisms of action of DOK2

DOK2 is a well-known tumor suppressor gene located on human chromosome 8p21.3 whose expression is downstream of tyrosine kinase, thereby affecting EGF-stimulated DOK2 phosphorylation. DOK2 operates through tyrosine kinase receptors, such as EGFR, PDGFR, and Her-2, with negative feedback regulating tyrosine kinase activity (Solarek *et al*.2019; Chen *et al*.2019; Ghanem *et al*.2014). For example, DOK2 interacts with EGFR. Through the action of an Src family kinase, DOK2 activates EGFR, which inhibits the EGF-P13K-Akt pathway, influencing the expression of related genes and proteins that inhibit cell proliferation and migration, promoting cell apoptosis and autophagy, which affects tumor development (Berger *et al*.2013; Van *et al*.2005). EGFR and RASA1 activation can also inhibit the EGF-RAS-MAPK-ERK pathway which inhibits cell division and affects the occurrence and development of tumors. In addition, DOK2 can recruit Csk family proteins that inhibit Src family kinase activity (Berger *et al*.2013). However, EGFR activation also induces DOK2

recruitment to the EGFR complex and DOK2-mediated recruitment of RASA1. DOK2 is also stimulated by epidermal growth factor, macrophage colony-stimulating factor (M-CSF), angiogenin-1 (Ang-1), interleukin-3 (IL-3), *etc*. DOK2 can be phosphorylated when stimulated by these factors (Suzu *et al*.2000; Master *et al*.2001; Jones *et al*.2003), as shown in Figure 2.

6. Summary

DOK2 plays an important role in many physiological functions, especially negative regulation of the T-cell signaling pathway and growth and development of hematopoietic progenitor cells. Moreover, it is associated with multiple diseases, such as leukemia, invasive tissue sarcoma, herpes simplex virus (HSV-1), peritoneal fibrosis, *etc*. In addition, many reports have demonstrated that downregulation of DOK2 is related to the occurrence of cancer. Berger et al. have confirmed that DOK2 is a target of genomic deletion and down-regulation in human lung cancer (54). Therefore, genetic testing prior to the occurrence of cancer should predict the risk of disease in patients. Similarly, we can also select DOK2 as a therapeutic target for cancer treatment to block its carcinogenic mechanism in advance. For prognosis related to surgery, DOK2 is also of considerable value as a reference. For example, in ovarian cancer, upregulation of DOK2 causes ovarian cancer cells to again be sensitive to platinum-based drugs (Fang *et al*.2018; Lum *et al*.2013). Therefore, to prevent, treat, and evaluate prognosis of cancer patients, DOK2 is an important target, but not the one and only most important.

Conflict of interest

The authors declare that there are no conflicts of interest.

Author contributions

PXC designed and supervised the study. LRM, SP, and MYY reviewed the references. LRM and SP wrote the manuscript. XSJ, WQQ, and YXL contributed to the Tables and Figures. PXC and CJ revised the manuscript. All authors approved the final manuscript,

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Ethical Statement

The study did not require ethical board approval because it was not part of a human or animal trial.

Abbreviations

DOK: downstream of tyrosine kinase; SHIP-1: inositol-5-phosphatase; Grb2: growth factor receptor-bound protein 2; EGFR: epidermal growth factor receptor; PDGFR: platelet-derived growth factor receptor; PTK: protein tyrosine kinase; Csk: C-Src tyrosine kinase; MAPK: mitogen-activated protein kinase; PTB: phosphorylation binding domain; NK: natural killer; IFN-γ: interferon gamma; GPVI: glycoprotein VI; TLR2: Toll-like receptor II; SFK: Src family kinase; LPS: lipopolysaccharide; TNF-α: tumor necrosis factor α; NO: nitric oxide; FcIR: Fc receptors; CD200FC: CD200 fusion protein; HSV-1: herpes simplex virus-1; PF: Peritoneal fibrosis; EMT: epithelial to mesenchymal transition; CML: chronic myeloid leukemia; MPD: myeloproliferative disease; ERK: extracellular-regulated protein kinase; PKB: protein kinase B; CLL: chronic lymphoblastic leukemia; CMML: chronic myeloid leukemia; ATLL: adult T-cell leukemia/lymphoma; KIf1: Kruppel-like factor; AML: acute myeloid leukemia; IGF-1: insulin-like growth factor

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Figure legends

Figure 1. DOK2 domain and potential function. The SH domain is at the DOK2 C-terminus. The Pleckstrin homology domain (PH) is connected at the N-terminal of DOK2 and promotes protein-protein interactions.

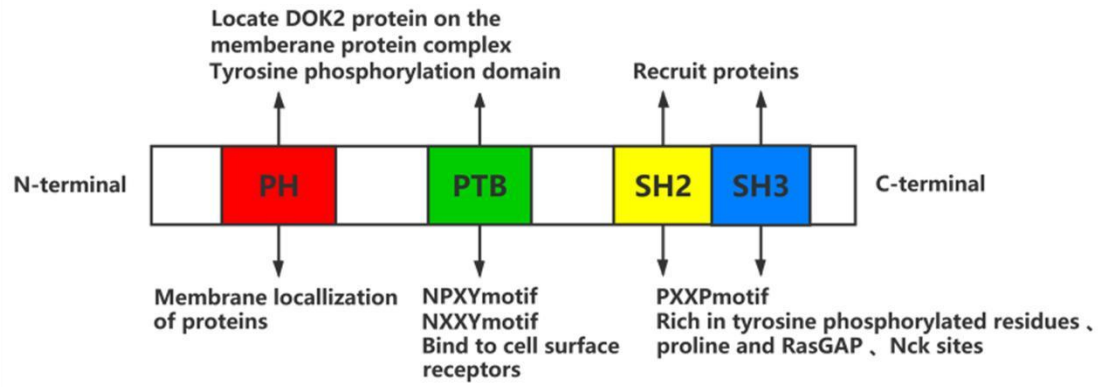

Figure 2. Potential role of DOK2 in tumorigenesis. DOK2 is regulated by

multiple factors such as EGF, PDGF, *etc*., which then regulate the Ras/PI3K signaling pathway causing abnormal cell proliferation and migration, ultimately leading to tumorigenesis.

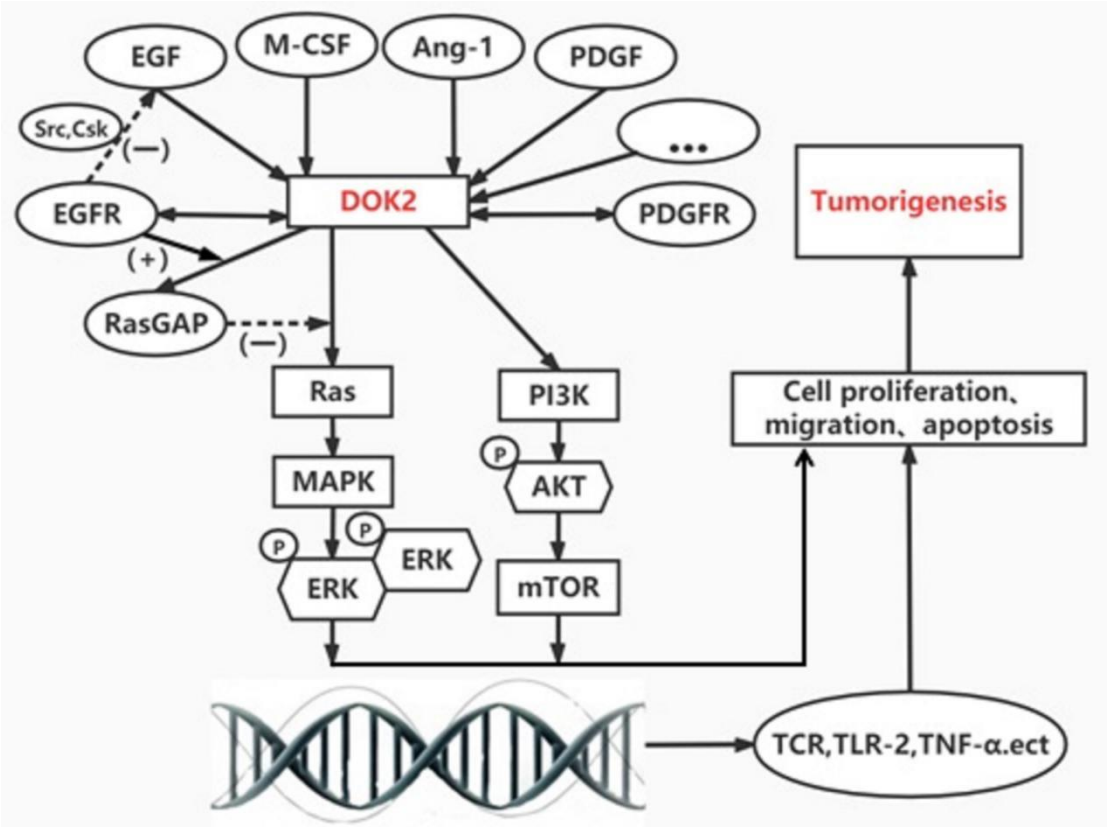

Table 1. Role of DOK2 and related factors in various physiological processes

Physiological processes	DOK2 and DOK2-associated factors
Cell fusion and proliferation	DOK2, DOK1, DOK3, IFN-γ
Regulation of the cell cycle of hematopoietic stem and progenitor cells	DOK2, DOK1
Regulation of platelets	DOK2, αIIβ3
Negative regulators of T cell receptor Signaling	DOK2, ZAP-70, SHIP-1, Grb-2, CD4, RasGAP, gp120
Tyrosine channel	DOK2, EGFR, RasGAP, TLR-2
Negative regulators of	DOK2, LPS, TNF-α, ERK

Lipopolysaccharide-induced signals	
FcIR	DOK2, FcIR
Modulation of memory CD8+ T cells	DOK2, DOK1,
CD200 and CD200R	DOK2, CD200, CD200R, GasGAP

Table 2. Role of DOK2 and related factors in various pathophysiological processes

Pathological process	DOK2 and DOK2-associated factors
Peritoneal fibrosis	DOK2, lncRNA(AK089579), JAK2/STAT3
Herpes simplex virus 1 (HSV-1)	DOK2, Vp11/12, DOK1
Glial inflammation	DOK2, TLR-2, IL-6
Leishmania infection	DOK2, DOK1, DOK3, GP63

Table 3. Genes or proteins associated with DOK2 and the related cancers

Cancer type	DOK2-associated gene or protein
Colorectal cancer	DOK2
Chronic myelogenous leukemia (CML)	DOK2, ERK, MAP, gp210
Adult T-cell leukemia (ATL)	DOK2, Tax
Erythroleukemia	DOK2, KIf1
Chronic lymphocytic leukemia	DOK2, Src
Aggressive histiocytic sarcoma (HS)	DOK2, DOK1, DOK3, M-CSF, GM-CSF
Lung cancer	DOK2, MAPK, Dusp4, EGFR
Renal cancer	DOK2, IGFs, Insulin
Breast cancer	DOK2, RASA1, MAPK, C-Src, Csk, EGFR
Glioma cancer	DOK2, NC
Lymphoma	DOK2, TCR

Astrocytoma	DOK2
Ovarian cancer	DOK2
Pancreatic cancer	DOK2
Gastric cancer	DOK2