Current research status of TNFAIP8 in tumours and other inflammatory conditions (Review)

JIANYU HUA[1], GUOHONG ZHUANG[1] and ZHONGQUAN QI[2]

[1]Organ Transplantation Institute of Xiamen University, Fujian Provincial Key Laboratory of Organ and Tissue Regeneration, School of Medicine, Xiamen University, Xiamen, Fujian 361102; [2]Medical College of Guangxi University, Nanning, Guangxi 530004, P.R. China

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Abstract. Tumour necrosis factor (TNF)-α-inducible protein 8 (TNFAIP8) is the founding member of the TIPE family. According to accumulating evidence, TNFAIP8 plays a pivotal role in the regulation of a variety of tumours, as well as inflammatory diseases. TNFAIP8 is suggested to act as an anti-apoptotic protein, affecting proliferation, metastasis, invasion, angiogenesis, drug resistance and immune response through multiple signalling pathways. From the clinical point of view, further studies should be required to confirm the prognostic value of TNFAIP8 and also clarify its possible contribution to the development of a novel therapeutic strategy to treat patients with tumours, including those of the breast, colon and lung, and inflammatory diseases. The present review focuses on the TIPE family member TNFAIP8 and describes the molecular mechanisms with regard to how TNFAIP8 could participate in the development of tumours as well as other conditions.

Contents

Correspondence to: Professor Guohong Zhuang, Organ Transplantation Institute of Xiamen University, Fujian Provincial Key Laboratory of Organ and Tissue Regeneration, School of Medicine, Xiamen University, 4221-122 Xiang-An-South Road, Xiamen, Fujian 361102, P.R. China
E-mail: zhgh@xmu.edu.cn

Professor Zhongquan Qi, Medical College of Guangxi University, 100 Da-Xue-Dong Road, Nanning, Guangxi 530004, P.R. China
E-mail: yxyyz@gxu.edu.cn

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1. Introduction

Inflammation is closely related to cancer, and inflammatory conditions increase the risk of cancer. To respond to the inflammation caused by a variety of factors, such as toxic chemical exposure, cells secrete the soluble pro-inflammatory cytokine tumour necrosis factor-α (TNF-α), which binds to TNF receptors I and II (TNFRI/II) on the cell surface, leading to the activation and phosphorylation of cytoplasmic inhibitor of κBα (IκBα) kinase. This binding allows nuclear factor-κB (NF-κB) to undergo nuclear translocation, thereby activating the transcription and expression of TIPE family members (1,2). This family includes four members: TNF-α-induced protein 8 (TNFAIP8), TIPE1 (TNFAIP8L1), TIPE2 (TNFAIP8L2) and TIPE3 (TNFAIP8L3). These four members are similar in structure but appear to play diverse roles in the progression of cancer and inflammatory responses (1). TNFAIP8, also known as SCC-S2, was the first member of the TIPE family to be discovered; it not only promotes tumour cell proliferation, survival and autophagy, and induces drug resistance, but also promotes tumour invasion, migration and angiogenesis, which are closely related to oncogenesis and tumour progression (3). Meanwhile, TNFAIP8 participates in the regulation of various other diseases; for example, it inhibits the inflammation response after injury and regulates cell apoptosis in inflammatory diseases. TNFAIP8 is also a risk factor for bacterial infection. This review will comprehensively summarize TNFAIP8-associated expression, regulation, functions and signalling pathways in diverse diseases.

2. TNFAIP8 structure

The novel gene TNFAIP8 was identified by analysing differentially expressed transcripts in head and neck squamous cell carcinoma (HNSCC) cell lines using mRNA differential display PCR (4). Importantly, the analysis of the TNFAIP8 open reading frame revealed a putative death effector domain (DED) at the amino terminus, which is significantly >25% similar to DED II in Fas-associated death domain-like interleukin (IL)-1β-converting enzyme-inhibitory protein and caspase homologous protein in humans and mice. DEDs play important roles in protein-protein interactions (2,5). For example, the death receptor TNFR1 interacts with

TNFR-associated death domain, the adaptor molecules Fas-associated death domain protein (FADD) and FADD-like interleukin-1β-converting enzyme leading to apoptosis (5). However, analysis of the TIPE2 crystal structure contradicts this assumption. Structural analysis shows that TIPE2 is composed of six antiparallel α helices, which differs from the known topological structure of DEDs by being a mirror image (6). Notably, the TNFAIP8 family has relatively high structural and sequence homology. Analysis of the human TIPE3 crystal structure (7) and TNFAIP8 from *Mus musculus* (mTNFAIP8)-phosphatidylethanolamine (PE) (8) revealed that in addition to the six α helices (α1-α6), mTNFAIP8-PE and TIPE3 have unique N-terminal α0-helices. A flexible short hinge motif exists between α0 and α1, while the remaining α helices (α1-α6) are folded into the TIPE2 homologous (TH) domain that is shared among TNFAIP8 family members. A highly conserved hydrophobic cylindrical cavity exists in the centre, and two long electron-dense regions exist in the cavity, which may be binding sites for phospholipid molecules. The studies on the crystal structures of TNFAIP8, TIPE2 and TIPE3 revealed that the TH domain of mouse TIPE family members could sufficiently bind to lipid messengers such as phosphatidylinositol. Phosphatidylinositol insert their lipid tails in the cavity, while the negatively charged head group forms a hydrogen bond with the positively charged amino acid residues on the cavity surface (7,9,10). Numerous hydrophobic cofactors or substrates are expected to bind inside the cavity in this way.

3. TNFAIP8 expression

The TNFAIP8 gene is located in the q23 region of chromosome 5, and its full-length cDNA clone was completely extracted and found to encode a cytoplasmic protein with a relative molecular weight of 21 kDa (1). Expression of TNFAIP8 was detected in the majority of normal tissues and cells, such as the spleen, thymus, lymph node, lung, gastrointestinal tract, uterus and prostate gland, but could not be detected in the brain (5). In particular, TNFAI8 was upregulated in malignant tumours, such as lung cancer, gastric cancer (GC) and chronic myelogenous leukemia (5). Compared with that in normal tissues and cells, differential expression of this protein in cancer is association with tumour development and progression. In recent years, several transcriptional variants of TNFAIP8 have been found. These variants are highly conserved in the C-terminus, but variable in the N-terminus. Among them, TNFAIP8 variant 2 (v2) is upregulated in a range of human cancer types and has been found to modulate cancer progression. The v1 variant is downregulated in the majority of cancer types, and its molecular weight differs from that of v2. The expression levels of v3-v6 are very low in normal tissues and cancer cells, which indicates that v1 and v2 of TNFAIP8 are differentially expressed in tumours, which might be related to their functions (8,11).

Expression of TNFAIP8 is regulated by the presence of a number of factors, including NF-κB, the p53 mutant K120R, liver insulin, chicken ovalbumin upstream promoter transcription factor (COUP-TFI), hypoxia-inducible factor-1α (Hif-1α), androgen, methylation and microRNAs (miRNAs/miRs).

As aforementioned, TNF-α binds to TNFRI/II, thereby activating the NF-κB pathway and inducing TNFAIP8 transcription. The K120R mutant of p53 binds to the TNFAIP8 locus on the p53 response element, activating the transcription of TNFAIP8 (1). Liver insulin can temporarily increase the expression of mTNFAIP8 (8). Genome-wide microarray analysis revealed that TNFAIP8 was the target gene of COUP-TFI, a transcription factor that participates in numerous biological processes. The TNFAIP8 promoter is co-occupied by the COUP-TFI complex, which is composed of nuclear receptor corepressors, transcriptional intermediary factor 1b and deleted in breast cancer 1. These factors mediate the transcriptional repression of TNFAIP8. TNF, which regulates the apoptotic pathway, alleviates the repression of the TNFAIP8 promoter by downregulating COUP-TFI expression (12). It is hypothesized that NF-κB can also interact with the COUP-TFI promoter through NF-κB-binding sites and suppress its expression, thereby weakening the inhibition of the TNFAIP8 promoter and ultimately inhibiting apoptosis by inhibiting caspase-8 activity (12). In addition, the expression of TNFAIP8 is important in the transcription factor Hif-1α signalling pathway, which is involved in cell survival, proliferation and migration (13). The TNFAIP8 promoter region possesses an androgen response element that can be induced by androgens synthesized by hormone-responsive prostate cells (14). Whole-gene analysis of androgen receptor (AR) in a long-term androgen-deprived prostate cancer cell line (LNCaP-AI) indicated that the TNFAIP8 gene was altered and potentially regulated by AR (15). GG2-1 (TNFAIP8) has been identified as a methylation target in a prostate epithelial cancer cell line (267B1) (16). In GC and osteosarcoma cells (MG-63 and U2OS), TNFAIP8 is a direct target of miRNAs, such as miR-9, miR-138 and miR-99a, which can decrease the expression of TNFAIP8 by interacting with its 3'-untranslated region (3'-UTR) (17-19).

4. Current studies of TNFAIP8 in tumours

TNFAIP8 is a crucial antiapoptotic and carcinogenic molecule, which is highly expressed in the cytoplasm of most tumour cells to mediate the occurrence and development of tumours. Specifically, TNFAIP8 plays an important role in promoting tumour cell proliferation, migration, invasion and angiogenesis, and inducing tolerance to chemotherapeutic drugs (20-23). Research on the link between inflammation and cancer has been ongoing for several years. Recent studies have shown that G protein-coupled receptors (GPCRs) are at the core of these two processes, regulating the two main pathways of NF-κB and STAT3 (23-26). NF-κB is regulated by a variety of GPCR signals and is associated with tumour metastasis by affecting cell migration. In addition, GPCR can also activate Ras, thereby activating the phosphoinositide 3-kinase (PI3K). This kinase can convert TIPE-anchored phosphatidylinositol 4,5-bisphosphate (PIP2) to TIPE-anchored phosphatidylinositol 3,4,5-trisphosphate (PIP3) (27). The lipid second messenger conversion was shown to be related to TNFAIP8 (9,10), which promotes the leading-edge formation of cells and recruits downstream molecules, such as Akt, which plays a major role in tumourigenesis (28), and Rac-GTP. TNFAIP8 in the cytoplasm inhibits Rac-GTP migration to the cell membrane to prevent leading-edge formation. In summary, TNFAIP8 participates in the directionality of cell migration by regulating

phosphoinositide signaling and Rac (10). Furthermore, PI3K also activates the STAT3 pathway, which is closely related to inflammation and cancer. However, the role of TNFAIP8 in the pathway for inflammation and tumour progression requires further research in specific diseases (23). In addition, previous studies showed that TNFAIP8 is not limited to regulating the proliferation and migration of tumours, but that it also mediated immune functions of $CD4^+$ T lymphocytes, promoting tumour progression. The expression level of TNFAIP8 in tumour-infiltrating $CD4^+$ T cells and $CD8^+$ T cells in patients with non-small cell lung cancer (NSCLC) was significantly lower than that in $CD4^+$ T cells and $CD8^+$ T cells in the peripheral tissues. Moreover, the expression of TNFAIP8 in advanced tumour-infiltrating $CD8^+$ T cells was lower than that in these cells at primary stages (22,29). TNFAIP8 may regulate the development of NSCLC by affecting the function of immune cells. The TNFAIP8 expression levels were increased in peripheral blood $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes in papillary thyroid cancer tissues, but there were no changes in the expression in monocytes or natural killer T cells. Additionally, the expression level of TNFAIP8 in tumour-infiltrating $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes was increased, indicating that TNFAIP8 may regulate tumour development by affecting immune cells (22,30). These results imply that TNFAIP8 often serves as a prognostic marker and potential therapeutic target for various malignant tumours. However, the research into TNFAIP8 in immunity is still limited, and further studies are required.

TNFAIP8 is a prognostic marker for tumours. Elevated expression of TNFAIP8 was found in patients with different cancer types, such as NSCLC (31), colon cancer (32), GC (33), epithelial ovarian cancer (EOC) (34), endometrial cancer (EC) (35), invasive ductal breast carcinoma (36), oesophageal squamous cell carcinoma (37), pN0 oesophageal squamous cell carcinoma (38), hepatocellular carcinoma (HCC) (39), diffuse large B-cell lymphoma (DLBCL) (40), clear cell renal cell carcinoma (ccRCC) (41) and papillary thyroid carcinoma (30), which are often significantly associated with poor prognostic features; for example, advanced tumour stage, lymph node metastasis, increased histological grade, poor survival time and tumour recurrence, thus implying its potential diagnostic and prognostic value.

Recent multivariate Cox regression studies showed that high TNFAIP8 expression is also an independent predictor. TNFAIP8 overexpression was correlated with lymphatic metastatic recurrence in patients without lymph node metastasis (pN0) who underwent Ivor Lewis oesophagectomy (38). Increased expression level of both TNFAIP8 and Ki-67 were independent factors of disease-free survival rates in patients with NSCLC and EC (31,35). Similarly, high expression of TNFAIP8 was associated with epithelial growth factor receptor (EGFR) expression levels and revealed the recurrence of pancreatic cancer (42). In gastric adenocarcinoma, TNFAIP8 is associated with a poor prognosis for intestinal-type gastric adenocarcinoma, but not for diffuse-type gastric adenocarcinomas, and is closely related to tumour invasion and the Lauren classification (43). In addition, serum CA72-4, a tumour marker, is associated with TNFAIP8 expression and is presumed to be associated with the early diagnosis and prognostic evaluation of gastric adenocarcinoma (44). Increased nuclear TNFAIP8 expression, which is regulated by karyopherin α2 (importin-α1), is an independent prognostic marker for recurrent prostate cancer (14).

Cancer susceptibility and tumour progression may be influenced by a single nucleotide polymorphism (SNP) of TNFAIP8, which further confers prognostic value to TNFAIP8. SNP analysis of TNFAIP8 revealed that the rs11064 variant of the GG genotype was associated with an increased risk of cervical cancer compared with that of the AA and AG genotypes, and G was identified as the risk allele (45). Given that the rs11064 SNP is located at the TNFAIP8 3'-UTR, the ability of miR-22 to target TNFAIP8 is decreased, which increases the expression of TNFAIP8 and upregulates the risk of cervical cancer and cisplatin resistance (45). The TNFAIP8 rs11064 and rs1045242 minor alleles were shown to be highly associated with the risk of EC in women in Heilongjiang Province, China; in particular, the G allele variation increased the risk of EC. In addition, rs11064 was associated with an advanced International Federation of Gynecology and Obstetrics stage, deep myometrial invasion and lymph node metastasis, suggesting that these SNPs are associated with the expression level of TNFAIP8 (46). The rs1045241 SNP in TNFAIP8 contributes to non-Hodgkin's lymphoma susceptibility in the Chinese population, and the T allele of the rs1045241 variant is associated with the risk of DLBCL and follicular lymphoma (47). With respect to these findings, TNFAIP8 can be used as a powerful prognostic marker and a potential therapeutic target for cancer.

TNFAIP8 regulates tumour growth, proliferation and drug resistance. Compared with that in adjacent non-cancerous tissues, TNFAIP8 is highly expressed in tumour tissues and promotes cell proliferation and tumour growth, which plays an important role in tumour progression, thereby affecting patient survival (33,48-50).

In a previous study, TNFAIP8 blocked TNF-α-induced apoptosis by inhibiting the enzymatic activity, but not the processing, of caspase-8 in a human fibrosarcoma cell line (HT1080I). The suppression of caspase-8 inhibited the cleavage of Bid (a substrate of caspase-8) and the activation of caspase-3. Crystallographic structural analysis of the TNFAIP8 family showed that the antiapoptotic effect may be achieved via the interaction between the hydrophobic cavity and the TH domain. Since the etoposide-induced apoptotic pathway is caspase-8-independent, it is not regulated by TNFAIP8 (2,9) (Fig. 1). TNFAIP8-silenced GC cells (BGC823) were treated with an anti-death receptor 5 monoclonal antibody to achieve antitumour effects by activating caspase signalling (51). Another study found that a mutation at the non-hotspot K120 of the tumour suppressor gene p53 deprived p53 of its ability to regulate tumour cell apoptosis, but resulted in the acquisition of novel oncogenic properties by inducing the transcription of TNFAIP8. The K120R mutant binds to the TNFAIP8 locus on the p53 response element to induce TNFAIP8 gene transcription and thereby promote cell survival. TNFAIP8 enable tumours expressing the K120R mutant to evade apoptosis by inhibiting caspase-8 enzymatic activity (52). Apart from caspase signalling, in Balb/c-3T3 fibroblasts, the coupling of Gαi and the short dopamine D2 (D2S) receptor stimulates

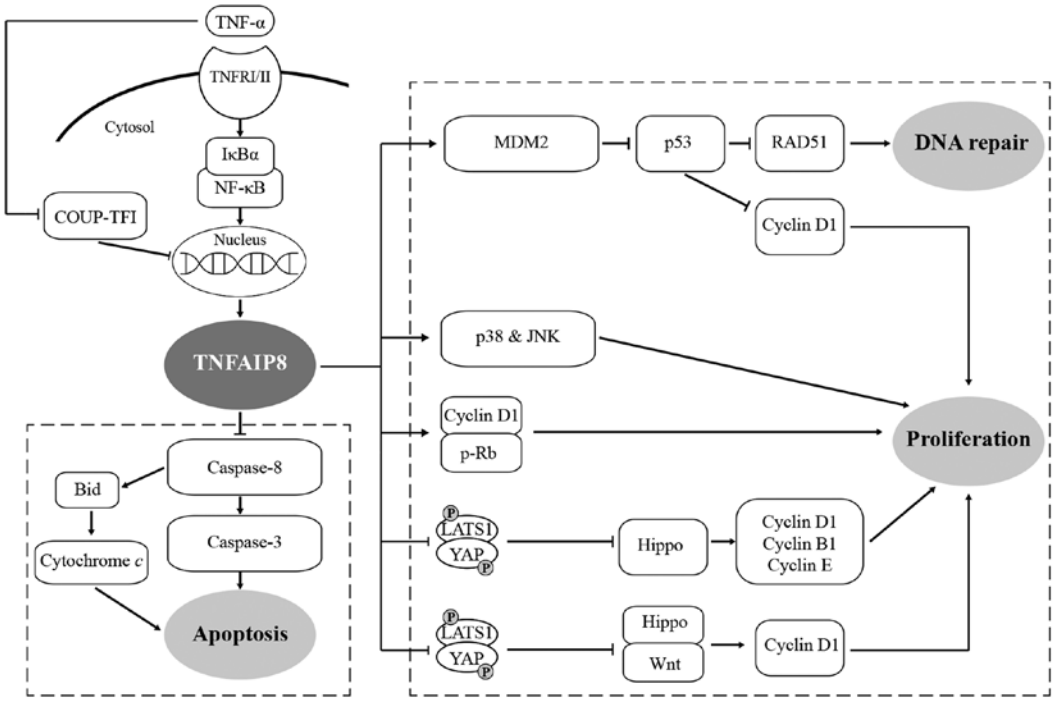

Figure 1. TNF-α induces the expression of TNFAIP8, activating proliferation and inhibiting the apoptosis of tumour cells through various signalling pathways, ultimately facilitating tumourigenesis and the development of tumours. ⊥, inhibition; TNFAIP8, tumour necrosis factor-α-inducible protein 8; TNFRI/II, TNF receptors I and II; NF-κB, nuclear factor-κB; LATS1, large tumour suppressor; YAP, yes-associated protein; COUP-TF1, chicken ovalbumin upstream promoter transcription factor; MDM2, murine double minute 2.

cell transformation and regulates cell proliferation, but is not involved in the MAPK-induced cell proliferation pathway (Fig. 2) (53). TNFAIP8 is a novel Gαi effector that may rely on the interaction between the TH domain and Gαi, suggesting that Gαi-TNFAIP8 coupling to the D2S receptor mediates transformation, and TNFAIP8 depletion does not affect other D2S-induced pathways, such as cAMP inhibition. D2S receptor signalling inhibits TNF-α-induced caspase-3/7 activation, but TNFAIP8 mediates Gαi-dependent D2S receptor signalling to prevent TNF-α-induced cell death via caspase-3/7-independent pathways. In summary, D2S/Gαi-TNFAIP8-induced signalling enhances the survival of oncogenic cells and promotes oncogenic transformation, and it may be possible to reduce tumour progression by blocking this caspase-independent pathway (53). Furthermore, the upregulated TNFAIP8 v2 in multiple human cancer types can interact with p53 to promote tumour development, promote DNA synthesis by maintaining the proliferating cell nuclear antigen levels in A549 cells, and inhibit p53-dependent cell cycle arrest by inhibiting p21 (the target of p53); however, the v2 variant inhibits cell cycle arrest only in cells such as U2OS and HCT116 cells, indicating that the v2 variant can be specifically regulated according to the cell type (11).

The hippo signalling pathway plays a crucial role in cell proliferation and apoptosis, and has become a hotspot of research. TNFAIP8 inhibits the phosphorylation of large tumour suppressor (LATS1) and yes-associated protein (YAP), and promotes YAP nuclear localization and TEA domain family member protein binding, which leads to the inhibition of the Hippo signalling pathway (39,54,55). Suppression of Hippo signalling can promote cell proliferation in lung cancer (54), EOC (55) and HCC (39). Inhibition of

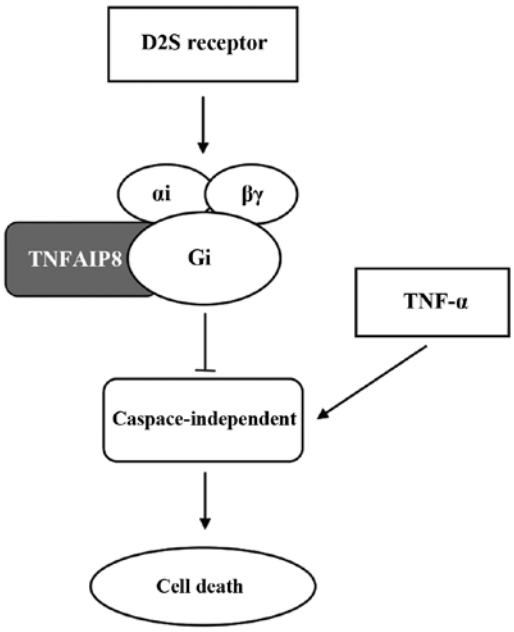

Figure 2. Gαi family-dependent dopamine-D2 short receptor signalling via coupling with TNFAIP8 inhibits TNF-α-induced cell death. ⊥, inhibition; TNFAIP8, tumour necrosis factor-α-inducible protein 8; Gi, heterotrimeric G proteins; αi, Gαi subunit; bγ, Gbg subunit; D2S, dopamine-D2 short.

this pathway in lung cancer cells (H460 and H1299) leads to increased expression of the downstream target genes cyclin D1 and cyclin-dependent kinases 6, and to decreased p27 expression. Similarly, inhibition of the Hippo pathway increases the cyclin B1 and cyclin D1 levels in EOC cells (A2780s), increases cyclin D1 and cyclin E levels, and downregulates p27

expression in HCC cells (HepG2 and SK-Hep1). Additionally, TNFAIP8 interacting with LATS1 may inhibit Hippo signalling, elevate YAP protein expression and subsequently activate Wnt signalling in colorectal cancer (CRC; H6T116), leading to increased expression of downstream cyclin D1 and promoting cell proliferation (56) (Fig. 1), suggesting that TNFAIP8 might be a new target for the prevention and treatment of cancer. By contrast, knockdown of TNFAIP8 in colon cancer cell lines (CACO2 and HCT116) results in reduced cell proliferation and inhibition of cell cycle progression by downregulating cyclin D1 and phosphorylated retinoblastoma (32) (Fig. 1). Knockdown of the TNFAIP8 gene affects antiproliferative and apoptotic genes such as IL-24, FAT tumour suppressor homolog 3 (*Drosophila*), latrophilin 2 and EPH receptor A3 in prostate cancer (PC-3), breast cancer (LM2-4175) and pancreatic cancer (PANC-1), indicating that new signalling pathways may be discovered in the future (13).

Elevated expression of TNFAIP8 promotes tumour cell proliferation-induced resistance of chemotherapeutic drugs, which results in a highly refractory nature and poor prognosis. Upregulated TNFAIP8 increases the risk of platinum resistance in EOC (57) and can interact with TAF-Iα to regulate cisplatin resistance in oesophageal cancer cells (EC-109/DDP and OE19/DDP) (58). Conversely, treatment with ionizing radiation or docetaxel following the decrease in expression of TNFAIP8 by an antisense TNFAIP8 oligonucleotide (LE-AS5) in a mouse model (14) and treatment with CDDP in TNFAIP8-downregulated oesophageal squamous cell carcinoma (37) resulted in increased apoptosis, inhibition of tumour growth and reversal of TNFAIP8-induced antitumour drug resistance. A similar result was observed in the chemotherapy treatment of tumour cells, which upregulated p53 and induced TNFAIP8 v2 expression, while v2-associated negative feedback regulation enhanced the p53-mediated resistance to apoptosis (11). In NSCLC cell lines (A549/cDDP), TNFAIP8 promoted tumour cell proliferation *in vivo* and *in vitro*, and increased cisplatin resistance, which is regulated by the murine double minute 2 (MDM2)/p53 pathway. TNFAIP8 upregulates the oncoprotein MDM2, increases p53 ubiquitination, and promotes p53 protein degradation, leading to the upregulation of RAD51 and cyclin D1 expression, and increased DNA damage repair and cell proliferation (59) (Fig. 1). Cisplatin treatment of TNFAIP8-silenced cervical carcinoma cells (HeLa) enhanced the activation of caspase-3/8 and mitogen-activated protein kinase phosphorylation, but inhibited the expression of B-cell lymphoma-2. These results suggest that TNFAIP8 promotes cell proliferation, colony formation and cisplatin resistance by negatively regulating the p38 MAPK signalling pathway (60). Furthermore, Liu *et al* (61) also found that an increased expression level of TNFAIP8 in RAW264.7 and EL4 cells, and exposure to ultraviolet irradiation or cisplatin inhibited the activation of caspase-3/9 and RARP, which revealed that TNFAIP8 plays an antiapoptotic role. In addition, the TNFAIP8 overexpression-induced activation of c-Jun N-terminal kinase and p38 kinase contributes to cell survival and facilitates tumour formation, but whether TNFAIP8 regulates the mitochondrial-mediated apoptotic pathway and the role of MEK remain to be investigated (61) (Fig. 1). Neoadjuvant chemotherapy (NACT) is an alternative therapy that can improve the efficacy of advanced OC surgical treatment. In a previous study, NACT treatment of tumour cells significantly decreased the expression of TNFAIP8, inhibiting the growth, proliferation and cell cycle of OVCAR-3 cells. Also, this treatment increased the level of LDH (indicating the degree of cell damage) and increased the sensitivity of cells to cisplatin, which improved the survival rates of the patients with OC (62). These findings might offer potential therapeutic targets for reversing the resistance of chemotherapeutic drugs by downregulating TNFAIP8 expression in patients with cancer.

TNFAIP8 also regulates cell survival and apoptosis through autophagy. Highly expressed TNFAIP8 mediates autophagy through the Akt/mTOR pathway and by targeting autophagosome protein 3 (ATG3) and ATG7, and regulates lipidation of LC3, thereby affecting cell survival and drug resistance (62-64). TNFAIP8 upregulation in PC-3 prostate cancer cells dysregulates the expression of multiple cell cycle-related proteins; it does not directly affect cell cycle progression but is associated with autophagy (63). Experiments have demonstrated that TNFAIP8 interacts with ATG3 to induce autophagy, increase autophagy effectors, such as microtubule-associated protein 1A/1B-light chain 3β (LC3βI/II), eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and Beclin-1, and stabilize the expression of sirtuin 1 and p62, which are related to autophagy regulation. TNF-α induces the expression of the autophagy marker LC3βI/II, which indicates that TNFAIP8 is involved in regulating TNF-α-induced autophagy, inhibits TNF-α-induced apoptosis by attenuating poly(ADP-ribose) polymerase (PARP) and caspase-3 cleavage, promotes the survival of prostate cancer cells, increases the drug resistance of cancer cells to the anticancer drugs docetaxel and doxorubicin, and promotes the progression of prostate cancer (63). Additionally, TNFAIP8-induced autophagy may contribute to neuroendocrine differentiation biomarkers in prostate cancer cells. However, the specific regulatory mechanisms need further study (63). Ectopic expression of TNFAIP8 in HCC induces the expression of the autophagy markers LC3βI/II, ATG3, 4E-BP1 and Beclin-1, and treatment of HCC cells (HepG2 and SK-Hep1) with TNF-α induces ATG3, ATG7 and LC3βII expression, and p-Akt and p-mTOR inhibition (64). These phenomena indicate that TNF-α induces the expression of TNFAIP8, inhibits the Akt/mTOR pathway, promotes the interaction of TNFAIP8 with ATG3 and ATG7 proteins, induces LC3 lipidation and autophagy, and promotes autophagy and cell survival of hepatoma cells. This results in the increased drug resistance of hepatoma cells to anticancer drugs, such as sorafenib and regorafenib, decreased PARP and caspase-3 cleavage, and decreased patient survival rates. This suggests that TNFAIP8 can increase autophagy and drug resistance in HCC cells and mediate the development of HCC, suggesting it as a new target for the diagnosis of early liver disease (64). Furthermore, TNFAIP8-knockdown in cisplatin-treated OVCAR-3 cells increased the expression of the autophagy marker proteins Beclin-1 and LCII, suggesting that TNFAIP8 modulates the response of OC cells to cisplatin by regulating the expression of autophagy-related proteins (62).

miRNAs interact with the 3'-UTRs of specific target mRNAs to regulate their expression at the post-transcriptional level. Studies have found that miR-9 is expressed at low levels in GC tissues and cell lines (MKN45 and MGC803), and that it targets the 3'-UTR sequence of TNFAIP8 and plays an

antitumour role by negatively regulating TNFAIP8 expression, resulting in reduced cell proliferation and increased sensitivity to antitumour drugs (17) (Fig. 1). miR-99a and miR-138 (18,19) in osteosarcoma (MG-63 and U2OS) and miR-155 in multiple myeloma (RPMI-8226 and MM.1S) also play the same roles (65), highlighting that miRNAs targeting TNFAIP8 represent a promising potential therapeutic target in the prevention and treatment of tumours.

Contrary to the aforementioned effect of promoting cell proliferation, glucocorticoids bind and activate glucocorticoid receptors, which bind to glucocorticoid response elements and regulate the *de novo* expression of genes, leading to the activation of apoptosis in thymocytes, thus promoting glucocorticoid-mediated cell death (Fig. 3). The upregulated gene expression of TNFAIP8 plays a functional role in glucocorticoid-mediated thymocyte apoptosis. Knockdown of TNFAIP8 expression has been show to protect thymocytes from glucocorticoid-induced cell death. These results indicate that the effect of TNFAIP8 on apoptosis depends on different environmental stimuli (66).

TNFAIP8 regulates tumour migration, invasion and angiogenesis. Tumour metastasis and recurrence are the leading causes of death in patients with cancer. As well as inhibiting apoptosis, elevated expression of TNFAIP8 in tumours promotes cell migration, invasion and angiogenesis by regulating the expression of the matrix metalloproteinase (MMP) family and vascular endothelial growth factor receptor 2 (VEGFR-2). This indicates the role of TNFAIP8 in tumour development and provides a basis for TNFAIP8-associated target cancer gene therapy. TNFAIP8 upregulation in breast cancer increased the expression of MMP-1 and MMP-9, but not MMP-2, which promoted cell migration and invasion. However, inhibition of endogenous TNFAIP8 expression with LE-AS5 resulted in the downregulation of VEGFR-2, which exerted the opposite effect (67) (Fig. 4). TNFAIP8 upregulation in EC increased MMP-9 expression (35). TNFAIP8-knockdown in pN0 oesophageal squamous cell carcinoma (Eca109) inhibited the expression of MMP-1 and MMP-9, but did not affect VEGFR-2 expression (38). TNFAIP8-knockdown in osteosarcoma cells downregulated MMP-2 and MMP-9 expression (18). TNFAIP8 silencing in hormone-refractory PC-3 prostate cancer cells caused the downregulation of MMP-1, MMP-2, MMP-9, membrane type 1 MMP and VEGFR-2. Additionally, microarray analysis indicated that TNFAIP8 and GDNF family receptor α1 might be involved in the regulation of tumour invasion (14). However, the invasion ability of NSCLC cells (A549 and H1299) was not regulated by the VEGFR-2, MMP-1 and MMP-9 pathways, but other regulatory pathways may exist (31). Furthermore, certain studies showed that an abnormal Hippo pathway is closely related to the recurrence and metastasis of cancer. TNFAIP8 promotes cell invasion in lung cancer (54) and HCC (39) by inhibiting the phosphorylation of LATS1 and YAP, promoting the nuclear localization of YAP and inhibiting the Hippo signalling pathway. This pathway leads to increased expression of the downstream target gene MMP-7 in lung cancer and HCC. TNFAIP8 inhibits Hippo signalling and thus activates Wnt signalling in CRC and promotes MMP-7 expression (56) (Fig. 4), suggesting that the effects of TNFAIP8 on the Hippo pathway influence tumour development and reveal

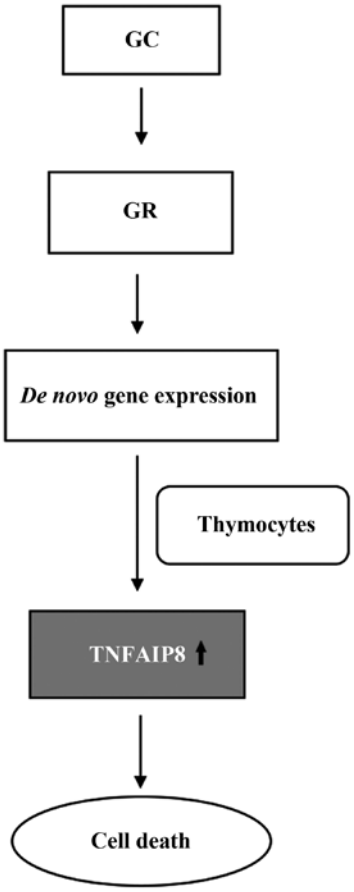

Figure 3. TNFAIP8 regulates GC-induced apoptosis in thymocytes. ↑, upregulation; GR, glucocorticoid receptor; TNFAIP8, tumour necrosis factor-α-inducible protein 8; GC, glucocorticoid.

the molecular mechanism involved. There are few studies on the regulation of TNFAIP8 with regard to invasion-associated gene expression. TNFAIP8 was silenced in oesophageal cancer cells (OE19), which resulted in the upregulation of TAF-Iα, while knockdown of TAF-Iα had the opposite effect, indicating that TAF-Iα is negatively correlated with TNFAIP8. TAF-Iα is a cytoplasmic protein that can bind to the mRNA encoding TNFAIP8. Silencing TAF-Iα in TNFAIP8-knockdown ECa cell lines reversed the TNFAIP8 downregulation-mediated inhibition of cell invasion and migration, suggesting that these factors can mutually regulate each other and that TNFAIP8 regulates cell migration and invasion through TAF-Iα (58). Additionally, TNFAIP8 is a multipronged target downstream of TNF-α, which regulates epidermal growth factor (EGF)- and IGF-1-stimulated migration through receptor tyrosine kinase signalling pathways in NSCLC cells (A549). EGFR is one of the most common growth factors implicated in numerous malignancies. A previous study showed that TNFAIP8-knockdown enhances the expression of sorted nexin 1 and upregulates the endosomal/lysosomal transport pathway, resulting in decreased EGFR expression, decreased EGF-induced phosphorylated extracellular signal-regulated kinase expression, reduced cell migration and increased sensitivity to EGFR mutation-selective tyrosine kinase inhibitors (68). In TNFAIP8-knockdown cells, the expression of IGF-1 binding protein 3 (IGFBP3) was shown to be increased, leading to a decrease in the IGF-1-induced expression of pIGF1R and p-Akt, decreased cell migration, and

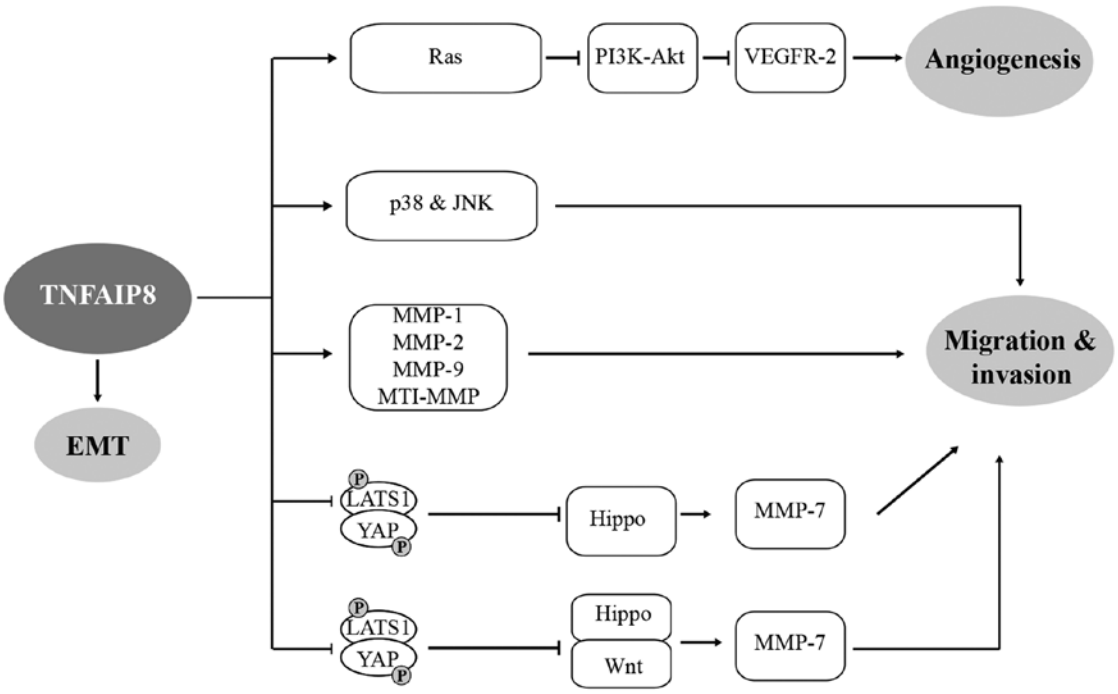

Figure 4. Role of TNFAIP8 in cell migration, invasion, EMT and angiogenesis through various signalling pathways, ultimately facilitating tumourigenesis and development of tumour. ⊥, inhibition; TNFAIP8, tumour necrosis factor-α-inducible protein 8; EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase. LATS1, large tumour suppressor; YAP, yes-associated protein; VEGFR-2, vascular endothelial growth factor receptor; MT1-MMP, membrane type 1 metalloprotease.

enhanced sensitivity to PI3K and Akt inhibitors, suggesting that targeting TNFAIP8 can inhibit adaptive responses and provided a rational strategy for the management of aggressive NSCLC (68).

Angiogenesis is an important step in tumour progression that relies on the release of angiogenic molecule. VEGF binds to VEGFR-2 to induce tumour angiogenesis and regulate CRC metastasis, which involves the PI3K-Akt signalling pathway (Fig. 4). Experiments showed that VEGFR-2 and TNFAIP8 were highly expressed in CRC tissues, and TNFAIP8-knockdown (HCT116) decreased the expression of VEGFR-2, the distribution of cell membrane microfilaments, migration and angiogenesis (69). Similar results were also observed in chicken chorioallantoic membranes and nude mouse models. TNFAIP8-knockdown was also found to inhibit the phosphorylation of Ras and PDK1, suggesting that TNFAIP8 regulates VEGFR2-mediated angiogenesis through the PI3K-Akt signalling pathway (69). Epithelial-mesenchymal transition (EMT) is known to drive tumour migration and metastasis. Silencing TNFAIP8 in ccRCC significantly reduced cell migration and invasion. Further experiments showed that high expression of TNFAIP8 decreased the expression of the epithelial markers E-cadherin and zonula occludens-1, and increased the expression of the mesenchymal markers N-cadherin and vimentin in ccRCC cells (769-P and ACHN). In addition, when TNFAIP8 was increased, TGF-β, zinc finger E-box-binding homeobox 1 and Slug expression was upregulated, while Snail expression was decreased. The opposite effect was observed when TNFAIP8 was silenced. This finding indicates that TNFAIP8 induces the migration and invasion of ccRCC by regulating EMT and is a potential therapeutic target for ccRCC (41).

5. Regulatory effects of TNFAIP8 in other conditions

Previous studies on TNFAIP8 have mainly focused on tumour-associated functions, but TNFAIP8 has also been shown to be involved in the regulation of other conditions, such as susceptibility to bacterial infection and suppression of inflammatory responses to injury. TNFAIP8 also modulates cell apoptosis in the resistance to inflammatory diseases. However, the research into TNFAIP8 in other diseases is still very limited, and further studies are required.

TNFAIP8 regulates bacterial infections. In a previous study of *Staphylococcus aureus*-induced sepsis, A/J mice were more susceptible than C57BL/6J mice, and this susceptibility was related to loci on chromosomes 8, 11 and 18. TNFAIP8 was differentially expressed between *S. aureus*-infected A/J and C57BL/6J mice, and this gene was shown to be closely associated with *S. aureus* susceptibility, as determined by quantitative trait locus analysis of chromosome 18 (70). Moreover, TNFAIP8-knockdown in *S. aureus*-infected RAW264.7 macrophages induced a decrease in cytokine IL-1β expression and an increase in granulocyte-macrophage colony-stimulating factor (GM-CSF). Similar changes were also observed in peritoneal macrophages from CSS18 mice, but not C57BL/6J mice, suggesting that TNFAIP8 is a strong candidate gene that contributes to *S. aureus* susceptibility *in vivo* and *in vitro* via the cytokines IL-1β and GM-CSF (70). Similarly, TNFAIP8 regulates the TNF-α-induced host cell apoptosis defence to *Listeria monocytogenes* infection through a Ras-related C3 botulinum toxin substrate 1 (Rac1) GTPase-dependent pathway (71). TNFAIP8-knockout mice resisted *L. monocytogenes* infection and exhibited a

decreased bacterial load; however, unlike the heightened immunity of TIPE2, TNFAIP8-knockout mice showed no resistance to extracellular pathogens and there was little difference in immunity, which suggested that the non-immune cells played an important role in protecting the host cells. TNFAIP8-knockdown in non-immune HCC cells (Hepa1-6) with TNF-α treatment increased the apoptosis of infected hepatocytes and induced the resistance to bacterial invasion. In addition, TNFAIP8-knockdown induced increased levels of p-Akt, Rac1-GTP and F-actin, and partially inhibited TNF-α-induced apoptosis by introducing a dominant-negative mutation of Rac1, indicating that TNF-α induces Rac1 and NF-κB activation; Rac1 activates the NADPH oxidase complex to produce reactive oxygen species (ROS), leading to apoptosis, while NF-κB promotes TNFAIP8 expression to inhibit ROS production by inhibiting Rac1 activation (71).

TNFAIP8 regulates inflammatory disease. TNFAIP8 regulates cell apoptosis to maintain colonic homeostasis. When TNFAIP8-knockout mice were administered water containing dextran sodium sulphate (DSS), they developed more severe colitis than wild-type mice, which manifested as weight loss, increased mortality rate, shorter colon lengths and enhanced inflammatory responses. TNFAIP8-deficient mice showed increased sensitivity to DSS-induced colitis, increased apoptosis of colonic epithelial cells and decreased cell proliferation, leading to the destruction of epithelial integrity. Increased transmission of symbiotic bacteria also caused a decrease in Akt activation, possibly mediating cell death by downregulating the PI3K-Akt signalling pathway, and the lack of TNFAIP8 in non-haematopoietic cells played a key role. It has been suggested that TNFAIP8 plays an important role in inflammatory diseases and can prevent colitis and maintain colonic homeostasis (72,73). Similarly, TNFAIP8-knockout mice are resistant to intestinal injury but have regeneration defects. This injury inhibition is regulated by a number of signalling pathways, such as that of PI3K/Akt/β-catenin. Ischaemia, ischaemia-reperfusion (I/R) and radiation-induced intestinal injury in TNFAIP8-deficient mice showed that their resistance to intestinal injury was mediated by non-immune cells, which also led to hyperproliferation, dedifferentiation and regenerative deficits in enterocytes. The regenerative programme requires YAP signalling, which activates Sca-1$^+$ (Ly6a) fatal-like cells and transiently induces Clu$^+$ revival stem cells (revSCs) in the intestinal epithelium (74). The dysregulation of regeneration was also observed in the TNFAIP8-knockout mice during DSS colitis. TNFAIP8-deficient mice lost the ability to modulate membrane phospholipid abundance and exhibited microbiome-dependent Akt activation and β-catenin accumulation, resulting in injury resistance, but decreased Sca-1$^+$ and revSC induction. Similar results were obtained in the colorectal cancer CMT-93 cell line (74). TNFAIP8 was also found to inhibit symbiotic microorganism-induced Akt activation by extracting PIP2 from cell membranes, reducing the amount of PIP3 and inhibiting PIP3-dependent signalling. It has been suggested that TNFAIP8 may play important roles in the treatment of colitis and colon cancer by regulating dynamic signalling to mediate the intestinal injury response and regenerative plasticity (74).

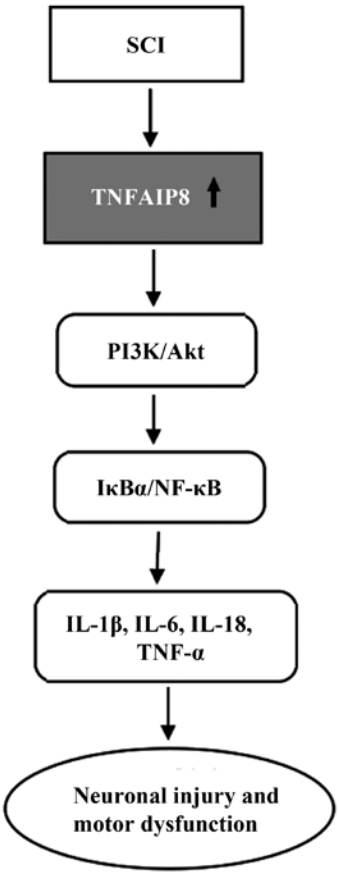

Figure 5. TNFAIP8 regulates the expression of pro-inflammatory cytokines through the promotion of IκBα/NF-κB and PI3K/Akt signalling in SCI. ↑, upregulation; TNFAIP8, tumour necrosis factor-α-inducible protein 8; PI3K, phosphoinositide 3-kinase; NF-κB, nuclear factor-κB; IκBα, inhibitor of κBα; SCI, spinal cord injury.

The high expression of TNFAIP8 in chronic pancreatitis tissues leads to the growth of inflammatory cells and enhances cell proliferation in inflammatory tissues (42). Plantar fascia lesions, such as those in plantar fasciitis and plantar fibromatosis, are associated with chr5:118704153:D in TNFAIP8 and with increased risks of disease development (75). Inhalation of the phosphodiesterase-4 inhibitor CHF6001 by patients with chronic obstructive pulmonary disease and chronic bronchitis after triple therapy effectively modulates pathophysiological pathways and downregulates pro-inflammatory cytokine genes, such as TNFAIP8, in sputum (76). The TNF-inducible protein TNFAIP8 is highly expressed in rheumatoid arthritis synovial fibroblasts and regulates apoptosis, proliferation and MMP-1 expression as an antiapoptotic gene (77).

TNFAIP8 regulates the inflammatory response. TNFAIP8 is highly expressed in both *in vivo* and *in vitro* mouse models of spinal cord injury (SCI) and time-dependently increases the expression of proinflammatory cytokines such as IL-1β, IL-6, IL-18 and TNF-α (Fig. 5). Suppression of TNFAIP8 expression alleviated the inflammatory response and cell viability by inhibiting the activation of the IκBα/NF-κB and PI3K/Akt signalling pathways in LPS-stimulated microglial BV2 cells, while pretreatment of these cells with SC-79 (an Akt activator)

reversed the inhibition of NF-κB signalling and pro-inflammatory cytokines (78). Similarly, TNFAIP8 deletion also ameliorated the inflammatory response, neuronal injury and motor dysfunction by inhibiting these signalling pathways in TNFAIP8-knockout mice after SCI, indicating that TNFAIP8 can regulate SCI through the Akt signalling pathway and that the regulatory effect of TNFAIP8 on inflammation was largely dependent on the PI3K-Akt signalling pathway. Multiple interactions may exist between TNFAIP8 and NF-κB depending on the environmental stimulus (78), and TNFAIP8 may be a novel target for developing effective treatment.

Allogeneic stem cell transplantation (aSCT) causes chronic graft-versus-host disease (cGVHD), and TNFAIP8 is a candidate molecular target of cGVHD and aSCT (79). In a previous study, allogeneic haematopoietic cell transplantation caused acute GVHD in the gastrointestinal tract, and TNFAIP8-knockout mice exhibited significantly exacerbated GVHD, weight loss and increased mortality rates (80). TNFAIP8 deficiency induced the downregulation of Ki-67 in non-haematopoietic and haematopoietic cells, increased epithelial cell apoptosis, destroyed the epithelial barrier integrity and increased symbiotic bacterial transmission, resulting in enhanced leukocyte infiltration and inflammatory responses, increased levels of proinflammatory cytokines such as IL-17A, TNF and interferon γ, and increased expression of chemokine (C-X-C motif) ligand 1, which contributes to exacerbating GVHD. This finding suggests that TNFAIP8 plays an important role in maintaining intestinal homeostasis and preventing complications associated with allograft transplantation (80). Controlled overexpression of TNFAIP8 might be an innovative therapeutic for GVHD progression. Diosgenin (DIO) has pharmacological effects on cerebral I/R. Proteomics analysis of brain tissues from DIO-treated rats with I/R showed that TNFAIP8 was a potential target and associated with autophagy and the inflammatory response. DIO treatment upregulated the I/R-induced decrease in TNFAIP8 expression, mediated the effect of TNFAIP8 on the regulation of autophagic activity and synergized with autophagy proteins in response to I/R injury (81).

6. Conclusions and prospects

TNFAIP8 is a novel protein that was discovered in the last decade; its family shares similar sequences and structures, and can bind to phosphatidylinositol through a conserved hydrophobic cavity, although each family member plays a different role. When considering the two long electron-dense regions inside the hydrophobic cylindrical cavity and positively charged residues near the entrance of the cavity, determining the manner of phosphatidylinositol binding will help in the further exploration of specific ligands or substrates. Moreover, the molecular mechanism of interaction, including the TNFAIP8-mediated antiapoptotic effect in the TH domain, still needs further elucidation. In addition, little research has been done on TNFAIP8 transcript variants, such as TNFAIP8 v2 and v1, which are known to be differentially expressed in most tumours, and whether they play different roles in different tumours. In recent years, TNFAIP8 has been shown to regulate not only apoptosis, autophagy, tumour migration and invasion, but also the proliferative activity of T lymphocytes, immune cell migration and other immune functions, thereby contributing to the development of new T lymphocyte-based therapeutic strategies. However, the specific mechanism of action is not as clear as that of TIPE2. Further studies are needed on how TNFAIP8 controls immune functions, whether immunoregulatory crosstalk exists among TIPE family members, and how T lymphocyte-targeted interventions affect tumours and inflammatory diseases. Additionally, the role of TNFAIP8 in cells depends on the cell type and the environment in which the cells are located. Although TNFAIP8 is known to regulate tumours and inflammatory diseases, the specific molecular mechanisms of their interactions and whether crosstalk exists between TNFAIP8 and signalling pathways, such as the Hippo, Wnt and PI3K/Akt/β-catenin pathways, remain unclear and need further elucidation. In conclusion, more in-depth studies on TNFAIP8 are needed to determine its prognostic, diagnostic and therapeutic roles in the clinic.

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Availability of data and materials

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Authors' contributions

Conceptualization and manuscript preparation were performed by JH. GZ and ZQ provided supervision and direction, and revised the paper. All authors have read and approved the manuscript. Data authentication is not applicable to this article.

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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