Progress in the understanding of C1q-like protein 4 (C1ql4) and its use in oncology

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Abstract

C1q and tumor necrosis factor (TNF)-related proteins (CTRPs) are a superfamily of proteins secreted in adipose tissues that are highly homologous to lipocalin. Previous studies have shown that this family has important biological functions in diseases such as metabolic disorders, cardiovascular diseases and inflammation in many types of tissues. C1q-like protein 4 (C1ql4) is one of the members of this family, which is mainly expressed in the testis and adipose tissue, and plays an important role in promoting angiogenesis, regulating lipid synthesis, inducting testosterone secretion, inhibiting ovarian granulosa cell apoptosis and myoblast fusion. This article reviews the current progress on molecular structure, tissue expression, and the main biological functions of C1ql4.

Keywords C1ql4; CTRP11; BAI3; Angiogenesis

Introduction

Immunity is the physiological process by which the body recognizes, eliminates and rejects all non-self molecules, and can be divided into innate and adaptive immunity. Among them, the complement system is an important component of innate immunity. C1 is the initial component of the classical complement activation pathway, which is mainly composed of three subunits: C1q, C1r and C1s. C1q can bind to a large variety of self-and non–self-ligands through its structural domain, linking the innate immunity initiated by the complement system with the adaptive immunity mediated by IgG or IgM.[1] In addition, C1q, also regulates a variety of physiological and pathological processes

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through the complement-independent activation pathway, such as pregnancy, tissue repair, and malignant tumor, etc.[2] The C1q family includes cerebellin (Cbln) and C1q-like protein (C1ql) subtypes. C1ql, also known as C1q and tumor necrosis factor-related protein (CTRP), consists of four subtypes C1ql1-4: C1ql1 (CTRP14), C1ql2 (CTRP10), C1ql3 (CTRP13), and C1ql4 (CTRP11). Studies have shown that C1ql4 has the properties of promoting angiogenesis and regulating fat synthesis, and has great protective potential in cardiovascular diseases, metabolic diseases and tumors. This article reviews the previous studies on the molecular structure, tissue expression and main biological functions of C1ql4.

1. Structure and receptor of C1ql4

Complement component C1q subunit-like protein 4 (C1q like 4, C1ql4), also known as C1q/TNF-related protein 11 (CTRP11), is an adipose-derived secreted protein and a member of the C1q/TNF superfamily of proteins. C1ql4 consists of 238 amino acid residues, including the signal peptide, N-terminal collagen domain and C-terminal globular domain.[3] Its structure is highly conserved during evolution and has a high degree of similarity in different species such as rat, mouse and human. The above species have exactly the same amino acid sequence of the spherical C1q domain (gClq).[4] (Fig.1).

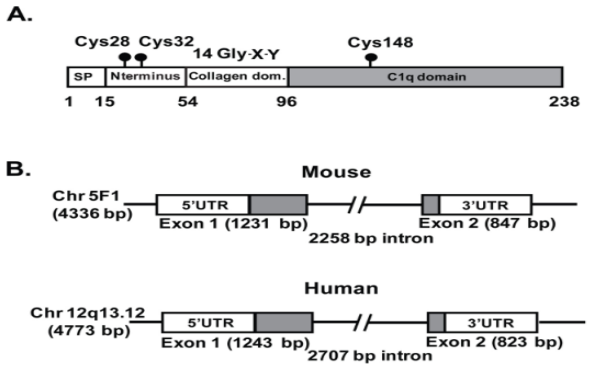

Figure 1. Identification of CTRP11. A: the deduced protein sequence of C1ql4 consists of four domains: a signal peptide (SP) for secretion, an N-terminal domain with two conserved Cys residues, a collagen domain with 14 Gly-X-Y repeats, and a C-terminal globular domain homologous to the immune complement C1q. The positions of Cys residues found in the mature protein are indicated with a ball-and-stick. B: Mouse C1ql4 is 4.3 kb long, is located on chromosome 5F1, and consists of 2 exons. Human C1ql4 is 4.8 kb long, located on chromosome 12q13.12, and also consists of 2 exons. UTR, untranslated region.

Brain-specific angiogenesis inhibitor3 (BAI3) is a high-affinity receptor for C1ql4.[5] Bolliger et al.[6] have used affinity chromatography and indirected immunofluorescence analysis to determine that C1ql4 can bind to the Thrombospondin1 repeat domain of BAI3 with high-affinity through the C1q globular domain. BAI is a subfamily of G-

protein-coupled receptors, mainly including BAI1, BAI2, and BAI3. BAI plays an important role in regulating angiogenesis, cell apoptosis, nerve growth, and cell-to-cell contact.[7] Studies have shown that the BAI3 is mainly restricted to brain regions (such as hippocampus, cerebral cortex, cerebellum and neurons), Leydig cells, vascular endothelial cells and myoblasts.[8] BAI is involved in neuromodulation, angiogenesis, and myoblast fusion. Liu et al. have detected the immunoreactivity of BAI3 in human umbilical vein endothelial cells (HUVECs), which has suggested that BAI3 may mediate C1ql4-induced angiogenesis.[9] Tan et al. have detected the developmental expression of BAI3 in mouse testes by qPCR.[10] Whether BAI3 is a receptor for C1ql4 in other cell types and plays a certain role still needs to be confirmed by clinical trials. If we can reveal the receptors on which C1ql4 works, it will greatly promote the exploration of its biological function, and it is possible to use it as a new potential clinical therapeutic target.

2. Expression distribution of C1ql4

Studies have shown that C1ql4 is highly expressed in testis and adipose tissue, and varies degrees in other tissues, such as the brain, kidney, placenta, ovary, heart, muscle tissue and spinal cord.[11] Wei et al., have established the cDNA library pool of C1ql4 in mouse and human tissues, which has the expression level of C1ql4 transcription product is the highest in the testis and adipose tissue of mice and humans. [3] PCR results have shown that C1ql4 is mainly expressed by vascular stromal cells, but not adipocytes. The expression of C1ql4 transcription product in brown adipose tissue is higher than that in white adipose tissue. In contrast, its expression in other tissues, including placenta, kidney, brain and other tissues, is relatively low. Interestingly, C1ql4 expression is higher in female rats than in male, and C1ql4 mRNA in the vascular components of the adipocyte matrix of female rats is 2.5 times that of male. Tan et al., have analyzed testicular tissues of pnd7, pnd21, and pnd56 mice, the results have shown that C1ql4 mRNA is expressed in seminiferous tubules and Leydig cells in the interstitium of the testis, and its distribution and expression levels are varied at different developmental stages.[10] Iijima et al., have studied the gene expression of the C1ql subfamily in adult and developing mouse brains by reverse transcription-polymerase chain reaction and high resolution in situ hybridization, and found that C1ql4 is slightly expressed in the brain tissue of adult mice. [4] Xue et al., have studied mouse ovarian granulosa cells isolated and cultured in vitro by RT-PCR, and detected the expression of C1ql4

in mouse ovarian granulosa cells.[12] Xue et al, have shown that C1ql4 protein is expressed in cardiac microvascular endothelial cells (CMECs) by RT-PCR, Western blot, and immunofluorescence analysis of rat CMECs.[13] Hamoud et al., have found that C1ql4 is expressed in myoblasts positive for the differentiation markers Pax3 and MyoD by using digoxigenin-labeled antisense ribose probes for in situ hybridization analysis on serial chicken embryo sections, and detected C1ql4 expression in the developing spinal cord.

3 Functions of C1ql4

3.1 Promotion of vascular renewal

Angiogenesis, the growth of new blood vessels from pre-existing vessels, including the processes of vascular dilation, endothelial cell permeability enhancement, basement membrane dissolution, endothelial cell migration and proliferation, lumen formation, endothelial cell differentiation and maturation. Many physiological and pathological processes are all based on angiogenesis, such as embryonic development, female reproductive cycle, normal development of the body, and repair after tissue damage. A better Understanding of the regulation mechanism of angiogenesis and regulating angiogenesis will provide new therapeutic options for many ischemic diseases, cardiovascular diseases and malignant tumors. Excessive angiogenesis is closely associated with the growth, invasion, and metastasis of malignant solid tumors. Anti-angiogenic therapy can normalize tumor blood vessels and improve the characteristics of the microenvironmental intermediate hypertension and hypoxia for tumor growth. These results can improve the efficacy of chemotherapy, radiotherapy, immunotherapy, and targeted therapy, and the prognosis of tumor patients. [14] Liu et al., have co-cultured C1ql4 protein expressed in a prokaryotic expression system with HUVECs, and found that gClql4 protein by HUVECs cells can promote the phosphorylation of c-Raf, MEK1/2, ERK1/2, and p90RSK.[9] Without the assistance of other factors in vitro, MEK1/2 inhibitor U0126 can block the endothelial cell migration and capillary formation induced by the C1ql4 globular domain (gC1ql4). This study shows that the ERK1/2 signaling pathway is involved in the angiogenesis of HUVECs induced by recombinant protein gC1ql4, and that C1ql4 promotes the migration of HUVECs and capillary formation in a dose-dependent manner. Above experiment also established a unilateral administration model of yolk sac membrane (YSM) to study the effect of C1ql4 on the growth of blood vessels in the YSM of chicken embryos, showing that the number of YSM vessels increased significantly after the

addition of gC1ql4 protein. Xue et al., have constructed the soluble non-fusion protein of gC1ql4, and found that gC1ql4 protein can promote tubule formation and cell migration of CMECs in rats.[13] After rat CMECs are treated with gc1ql4 protein, the phosphorylation process of ERK1/2 is activated. MEK1 inhibitor U0126 has an inhibitory effect on tubule formation and cell migration in CMECs, indicating that the gC1ql4 protein prepared by prokaryotic expression in vitro could promote angiogenesis of CMECs by activating the ERK1/2 signaling pathway. These experimental data have shown that C1ql4 stimulates new blood vessel growth by activating the ERK1/2 signaling pathway. The angiogenesis effect of C1ql4 may provide a new therapeutic idea for ischemic injury vascular repair, tissue and organ regeneration, and wound healing. The angiogenesis effect of C1ql4 may also provide a new interference target for tumor anti-angiogenesis therapy.

3.2 Induction of testosterone secretion

C1ql4 can induce testicular interstitial cells to secrete testosterone, and increase the expression of the steroidogenic acute regulatory protein (StAR) and steroidogenic enzymes. Cholesterol is the precursor of testosterone synthesis, and StAR is the key factor that promotes its transfer from the outer mitochondrial membrane of Leydig cells to the inner membrane. Tan et al., have found that C1ql4 mRNA and protein levels in mouse testis increase progressively from birth to adulthood.[10] In that study, the authors have shown that C1ql4 is expressed mainly by Leydig cells and seminiferous tubules in the testis. Separately, C1ql4 has developmental reactivity and is regulated by luteinizing hormone. This study has shown that recombinant C1ql4 activated c-Raf, MEK1/2, ERK1/2, MSK-1, and cAMP/PKA/cAMP response element-binding protein signaling cascades, And inducing the stimulating effect of testosterone secretion in Leydig cells, C1ql4 was accompanied with increasing in the expression of StAR and steroid-producing enzymes. Using in vitro TM3 Leydig cell models, the researchers demonstrated the presence of an unknown receptor in Leydig cells in addition to BAI3, that interacted with C1ql4 to activate the c-Raf, MEK1/2, ERK1/2, MSK-1, and cAMP/PKA/cAMP signaling pathways, upregulate StAR expression and stimulate testosterone production. At present, there are few studies on the regulation of StAR expression by C1ql4 in Leydig cells, and further research may provide an experimental basis for clinical research on diseases such as abnormal testosterone metabolism and malignant tumors of the reproductive system, and new ideas for related research on the pathogenesis of various diseases.

3.3 Regulation of myogenic cell fusion

The combination of C1ql4 and BAI3 is a spatiotemporal negative signal for myoblast fusion. Myogenic fusion is a specific type of cell fusion, which is a key process to promote muscle growth and repair muscle fiber tissue after injury. BAI3 is a receptor that coordinates myogenic fusion through Elmo/Dock1 signals, but the mechanism regulating its activity remains unclear. Hamoud et al.[8] have constructed a BAI3 binding defect C1ql4 mutant (sugar wedge mutant: C1ql4 GW), performing after the loss of interaction between C1ql4 GW and the BAI3 extracellular structural domain, and have confirmed that the inhibitory function of C1ql4 on myogenic cell fusion is due to its interaction with BAI3. To further confirm the function of C1ql4 as a BAI3 ligand to inhibit fusion, researchers constructed soluble fragments of each domain of the extracellular portion of BAI3. This study has shown that the CUB of BAI3 is the minimal and basic region carrying C1ql4 binding activity. By constructing a BAI3 mutant without C1ql4 binding activity (Flag-BAI3 ΔCUB) for differentiation experiments, it is directly evaluated that the binding of C1ql4 and BAI3 will inhibit myocyte fusion. The above data suggest that the activity of BAI3 is regulated by C1ql4 during myogenic cell fusion. The inhibitory effect of C1ql4 on myoblast fusion may provide new ideas for studies related to Skeletal muscle development and repair after injury.

3.4 Regulation of fat synthesis

C1ql4 plays an important role in the regulation of adipose tissue homeostasis. Adipose tissue is regarded as an endocrine organ with high metabolic activity, which can secrete hormones and transport them through the blood to reach the target tissues. Adipose tissue can also regulate physiological processes such as steroid metabolism, adipogenesis, angiogenesis, extracellular matrix reorganization, and body temperature maintenance. Wei et al., have treated 3T3-L1 pre-adipocytes with a culture medium containing C1ql4 and found that the medium containing C1ql4 inhibited PPAR-β, adipogenesis and lipid droplet biogenesis genes, and the expression of lipoproteins and markers are significantly inhibited.[3] Of these, adiponectin, an adipocyte-specific protein, has shown the most dramatic reduction. These results have shown that C1ql4 inhibits adipocyte differentiation through paracrine. At the same time, it is found that overexpression of C1ql4 can inhibit the mitosis of 3T3-L1 cells. Moreover, C1ql4 can inhibit adipogenesis by inhibiting the ERK1/2 signaling pathway of 3T3-L1 cells, and reduce the expression of two transcriptional regulators PPAR-γ and C/EBP-α.[15] C1ql4 is highly expressed in adipose

vascular interstitial cells and regulates adipocyte differentiation by paracrine. At the same time, C1ql4 also provides the possibility of directly regulating the angiogenesis process in the adipose interstitium.[3] The dysfunction of adipose tissue is the basis of the mechanism of metabolic diseases. Understanding the molecular and cellular mechanisms by which adipose tissue regulates systemic metabolism and causes metabolic diseases will influence the treatment of related metabolic diseases. C1ql4 plays a certain role in the regulation of fat synthesis, but whether this role is important in the physiological regulation and pathological state of systemic lipid metabolism still needs further research.

3.5 Inhibition of apoptosis of ovarian granulosa cells

C1ql4 can regulate the expression of apoptosis genes and inhibit the apoptosis of mouse ovarian granulosa cells. Xue et al., have isolated mouse ovarian granulosa cells and found that C1ql4 is expressed in mouse ovarian granulosa cells by RT-PCR.[12] In order to obtain high-quality gC1ql4 protein, the researchers also have constructed a recombinant expression vector containing gC1ql4, which is placed in E. coli BL21 (DE3) for low-temperature induced protein expression and purified by Ni2+Chelating Sepharose affinity chromatography column to obtain the globular structural domain protein of C1ql4. The protein has been co-cultured with mouse granulosa cells, and the apoptosis of granulosa cells has been detected by Hochest 33258 staining and the changes of Bcl-2 and Bax mRNA expression have been examined by qPCR. These studies have indicated that C1ql4 can inhibit the apoptosis of mouse ovarian granulosa cells by significantly up-regulating the expression of Bcl-2 and down-regulating the expression of Bax. The apoptosis of granulosa cells can affect the development and ovulation process of the egg, which is a potential mechanism of follicular atresia.The inhibition of granulosa cell apoptosis by C1ql4 may provide a reference for the study of the pathogenesis and treatment of premature ovarian failure, polycystic ovary syndrome, hormone-related cancers and other female reproductive system diseases.

4. Prospect

Studies have shown that Clq14 plays a very important role in promoting angiogenesis, regulating metabolism and endocrine, among them, promoting angiogenesis has great potential in clinical applications. Angiogenesis plays an extremely important role in the occurrence and development of malignant tumors, metabolic diseases, cardiovascular-related diseases and ischemic diseases.

Table 1: Summary of the C1ql4 study

Models	Target	Role	References
HUVECs、YSM、CMECs	ERK1/2	Promotes angiogenesis	[11] [14]
TM3 Leydig cells	c-Raf、MEK1/2、ERK1/2、MSK-1 and cAMP/PKA/cAMP	Up-regulation of StAR expression and stimulation of testosterone production	[10]
3T3-L1 cells	ERK1/2	Inhibits fat production	[3]
mouse ovarian granulosa cells	Bcl-2 and Bax	Inhibition of apoptosis in mouse ovarian granulosa cells	[13]

During the occurrence and development of malignant tumors, tumor cells can secrete high levels of vasophile factors and generate abnormal vascular networks, which affect tumor growth and metastasis. In the vascular complications of common diseases and cardiovascular diseases, the decrease of angiogenesis can cause the occurrence and even progression of various ischemic and hypoxic diseases. Therefore, the study of drugs affecting angiogenesis has become a major research focus on the treatment of tumors, prevention and treatment of cardiovascular and metabolic diseases. However, our present understanding of Clq14 is still very limited, It is hoped that more in vivo and in vitro experiments can be used to further clarify the internal mechanism and function of Clq14, and provide new ideas for the diagnosis and treatment of clinical diseases as much as possible.

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