MINI-REVIEW

Cancer Research Advances Regarding the CKLF-like MARVEL Transmembrane Domain Containing Family

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Abstract

The CKLF-like MARVEL transmembrane domain-containing family (CMTM) is a novel family of genes first reported at international level by Peking University Human Disease Gene Research Center. The gene products act between chemokines and the transmembrane-4 superfamily. Located in several human chromosomes, the CMTMs CKLF and CMTM1 to CMTM8 may be unregulated in tumors and act as potential tumor suppressor genes with important roles in the immune, male reproductive and hematopoietic systems. In-depth studies in recent years established a close relation between CMTMs and tumorigenesis and metastasis. The CMTM family has a significant clinical value in diagnosis and treatment of diseases linked to tumors and the immune system.

Keywords: CMTM family - gene - chemokine-like factor - tumor - diagnosis -treatment

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Introduction

CKLFSF is a new human gene family which was first reported at international level by Peking University Human Disease Gene Research Center. Wenling Han, a professor in Peking University, cloned a new CKLF1 successfully by utilizing Suppressive subtraction hybrization (SSH) from PHA-stimulated U937 cell (Han et al., 2001). CKLF1 has other three variants named CKLF2, 3, 4 (Han et al., 2001).CKLF2 is a whole-genome production of CKIF. Based on the nucleic acid and protein sequence of CKLF2, researchers used the technology of bioinformatics and RT-PCR to clone CMTM1-8, consisting of 9 genes-CKLF and CKLFSF1-8 (Han et al., 2001; Han et al., 2003; Zhong et al., 2006; Bu et al., 2008; Li et al., 2011). In 2005, Human Gene Nomenclature Committe (HGNC) recommended denominating CKLFSF1-8 as CMTM1-8 according to their structural features (Han et al., 2003; Zhong et al., 2006). Even better, this family plays a crucial role in immune system, male reproductive system and hematopoiesis system. With the in-depth study in recent years, researchers found the close relation between CMTMs and the genesis, development, metastasis of tumors (Han et al., 2003; Zhong et al., 2006). Therefore, CMTM family has a significant clinical value in diagnosis and treatment to the diseases linking to tumors and immune system. In this paper, we announced a summarization based on CMTMs' structural features, biological functions and the connections related to tumors.

Brief Introduction of CMTM Family

As frequently deleted in multiple tumors, CKLF and CMTM1-4, located in a gene cluster on 16q22.1, is supposed to be tumor suppressor genes (TSG). CMTM1, which has seven exons and six introns and two transcription start sites, is highly expressed in male reproductive systems (Bu et al., 2008). In CMTM family, CMTM3 is most closely related to CMTM5, with 42% amino acid identity. Among this family, CMTM5 is located in 14q11.2 separately. In normal adult tissues and normal epithelial cell line, CMTM3, CMTM5 and CMTM7 are broadly expressed, but CMTM3 and CMTM5 are downregulated or silenced in many kinds of tumor cell lines (Li et al., 2011; Bu et al., 2008; Wang et al., 2009). CMTM4 is the highly conserved gene which has three RNA splicing forms designated as CMTM4-v1, CMTM4-v2 and CMTM4-v3.However, only CMTM4-v1 and -v2 could be found in multiple tissues and cell lines. It has been reported that CMTM4-v1and-v2 are widely expressed in normal adult tissue such as the cytoplasm the cell membrane (Plate et al., 2010).

CMTM6-8 is from a gene cluster on chromosome 3p22. CMTM7 is broadly expressed in normal tissues but frequently silenced or under-expressed in some cancer types. It has been detected that Promoter methylation

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and loss of heterozygosity (LOH) are the mechanisms contributing to CMTM7 under-expression. The inhibition of cell proliferation and motility result from Ectopic CMTM7 expression (Li et al., 2014). In some cell lines, under-expression of CMTM8 can induce cell apoptosis through a mitochondriamediated pathway and function as a negative regulator of EGF-induced signaling (Li et al., 2014).

CMTM Family and Cancer

CMTM1

CMTM1 is widely expressed in normal lung tissue, but downregulated in non-small cell lung cancer (Bu et al., 2008). This indicates that it is a potential tumor suppressor gene. CMTM1-v17,one of RNA splicing forms, is highly expressed in both normal prostate tissue and prostate carcinom originated cell lines. This indicates it may be a new potential corepressor of androgen receptor and recruit histone deacetylase to exert its tumor suppression function (Bu et al., 2008).

CMTM3

It was found that the aberrant promoter CpG methylation will lead to gene silencing. Through epigenetics mechanism, the promoter of CMTM3 was methylated frequently, which can be authenticated in many cancer cells and some primary tumors. Under these circumstances, the transcription of p53 was inhibited, and the expression of p21 and the function of tumor suppresser gene decrease. In addition, the activation of caspase 9.8.3 is often diminished and PARP division process is reduced. The demethylated medicine can reverse this condition. The study at mRNA and protein level indicated that the tumor suppresser gene CMTM3 can induce cell apoptosis by blocking generation cycle G2.This function may be correlated with its conserved region MARUEL (Li et al., 2014).

After the tissue was infected by CNE2, the amount of cells which contains CMTM3 decreases dramatically. Studies showed that the restoration of CMTM3 which is expressed ectopicly induced chromosome condensation, externalizing phosphatidylserine and activating Caspase-3. This can not only prevent the formation and the existence of cancer cells effectively, but also induce cell apoptosis by activating caspase 3 (Yang et al., 2013).

CMTM3 is highly expressed in the male reproductive system, with the highest expression level in the testes. Studies showed that the methylation of a single CpG site located within the Sp1/Sp3-responsive region of the core promoter led to the under-expression of CMTM3 in clinical tumor tissues. It was also indicated that formation and proliferation was inhibited because of ectopic expression of CMTM3 (Li et al., 2011).

CMTM3 is connected with the formation and development of renal clear cell carcinoma (ccRCC) strongly. Previous studies showed that in normal adult renal tissue CMTM3 expressed strongly positive or moderate positive but was down-regulated or silenced in ccRCC tissues. Moreover, CMTM3 protein was undetectable in most detected ccRCC and cell lines. Therefore the prerequisite for the development of ccRCC may be the depletion of CMTM3.It was indicated in studies that CMTM3 might have effect on primary ccRCC .However, restoration of CMTM3 plays an important role on inhibiting formation, proliferation and migration of 786-0 cell's colony and induces the inhibition in vitro anchorage-independent growth. However, no aberrant promoter methylation was detected in ccRCC tissues. The result indicated that down-regulation of CMTM3 might have a connection with other epigenetic or genetic mechanisms, unlike aberrantly methylated in many tumors. .In addition, its epigenetic silencing might cause renal tumorigenesis (Xie et al., 2014).

Findings from Peking University showed that CMTM3 strongly affected migration and invasion of gastric cancer. Restoration of CMTM3 played important roles in AGS and SGC-7901 cell migration and invasion. In vivo experiments, it was indicated that CMTM3 strongly deterred peritoneal disseminated metastases. Further studies indicated that restoration of CMTM3 might induce decrease on the expression of mmp2 and the phosphorylation of erk1/2. In addition, through immunohistochemical staining, the studies showed that in gastric cancer tissues the expression of CMTM3 was significantly down-regulated comparing with that in normal mucosae and was strongly connected with gender, tumor depth, stage, and histological grade. Furthermore, in gastric cancer patients, CMTM3 expression was correlated with prognosis, and was an essential indicator for prognostic (Su et al., 2014).

In Zhang H's study, it is also showed that abnormal expression of CMTM3 was associated with oral squamous cell carcinoma. Due to hypermethylation in promoter, CMTM3 is downregulated and which has a strong connection with the migration and proliferation of OSCC cells. Furthermore, this study indicated that CMTM3 could predict the survival of OSCC patients as an independent prognostic factor (Zhang et al., 2014).

CMTM4

CMTM4,which has three RNA splicing forms called CMTM4-v1, -v2, and-v3, is a highly conserved gene in the CMTM gene family. CMTM4-v1 and -v2 are widely expressed in many types of tissues, and they are also distributed in the cytoplasm and on the membrane surface of the cell (Peng et al., 2009).The positive expression rate of CMTM4 is 31.7% in the mucosal tissue of esophageal and cardia cancer, but 100% in the normal mucosa tissue adjacent to carcinoma. The difference has statistical significance ($P<0.001$) (Peng c,et al., 2009).The gender, pathologic types, pathologic differentiated degree and clinical stage have no obvious correlations with the expression of CMTM4 ($p>0.05$) (Peng et al., 2009). By mediating G2/M phase retardation, the overexpression of CMTM4-v1 and -v2 not only suppress the growth of synchronized and non-synchronized Hela cells, but also suppress the clone growth of Hela cells. The changes of CMTM4's expression probably have an influence on Hela cells' cycle and fission. Sequentially it plays a significant role in the cell cycle regulation and cell proliferation (Plate et al., 2010).

The new research showed that the downregulation of CMTM4 was found in ccRCC tissues and in the 786-O and A498 ccRCC cell lines. 786-O cell growth and migration were inhibited after restoring the expression of CMTM4. Also, in nude mice, up-regulated CMTM4 suppressed ccRCC xengraft growth suggesting consideration during treatment (Li et al., 2011).

CMTM5

CMTM5 plays a significant part in inhibiting tumor, and it is widely expressed in most normal tissues. The silence of promoter is a kind of mechanism to inhibit its expression. Treatment with a methyltransferase inhibitor, 5-Aza, alone or combined with trichostatin A, a histone deacetylase inhibitor, can restore the expression of CMTM5 in the cancer cells, inhibit the colony formation of tumor cells, and then suppress proliferation and metastasis of tumor cells effectively. This suggests that CMTM5 probably make contribution to cancers as a tumor suppressor gene (Li *P*, et al., 2011).In addition, the expression of CMTM5 is closely related to tumor differentiation degree in epithelial ovarian cancers: the percentage of lost CMTM5 expression is 14.63% in well-differentiated and moderately differentiated cancers, while it increases to 46.15% in poorly differentiated cancers. These results suggest that the loss of CMTM5 expression is closely related to the progress and invasion of tumors (Li et al., 2011).

The new research indicated that CMTM5 might be used as a new prognostic indicator for oral squamous cell carcinoma (OSCC) in the clinic. Researchers observed that the expression of CMTM5 was closely related to the age of OSCC patients. In young patients, the decrease or abnormality of CMTM5 expression is very common. That suggests the inactivation of CMTM5 medicates the early tumor and plays an important part in the pathological changes in young OSCC patients. Moreover, CMTM5 participates in some signaling pathways related to cancer development, which might be an important mechanism of its functioning (Zhang et al., 2014).

Another research have found CMTM5 might be a potential therapeutic target for clinical diagnosis in prostate cancer (PCa).Their new research indicated the expression of CMTM5, compared with BPH tissues, was reduced in PCa tissues and cells ,which also related to the Gleason score. Study showed the restoration of CMTM5 may be contributed to the tumor growth inhibition in vivo (Xiao et al., 2015).

CMTM7

Bioinformatics analysis and Gene Cards database show that the CMTM7 is broadly expressed in normal tissues and epithelial cell lines ,but frequently silenced or downregulated in some cancer lines and primary tumor (Liu et al., 2013). And the mRNA level of CMTM7 showed a high state in tumor, compared with the other groups (Xie et al., 2014). Researchers who carried out the PCR of multiple differentials genomic DNA found epigenetic variation had not been expressed, and its reason were promoter methylation and LOH expression (although the possibility of hemizygous deletion could not be excluded). What's more, the loss of chromosome 3p allelic in esophageal cancer also showed that genes are involved in the down-regulation of CMTM7 expression mechanism (Liu et al., 2013).

By inducing G1/S cell cycle arrest, upregulating p27, downregulating CDK2 and K6, the CMTM7 promotes the epidermal growth factor receptor (EGFR) internalization and inhibits the cell proliferation and metastasis. From the results of the activation of AKt and ERK, researchers found that in KYSE180 cells the CMTM7 down-regulates the phosphorylation of Akt, inhibits the ERK activation, thereby inhibiting EGFR activation in the p13K/Akt downstream target (Liu et al., 2013). Above research suggests that CMTM7 may be a tumor suppressor factor.

Miyazaki also reported in mouse B lymphoma Ba117 cells, through the activation of ERK and C-JnnN–terminal Kinase, CMTM7 can combine with BLNK to negatively regulate BCR endocytosis. As a former B cell leukemia tumor suppressor factor, BLNK can lead upregulation of the expression of P27 and inhibit cell division to G1-S phase transition. As everyone knows, G1-S conversion is the key node in the cell cycle (Miyazaki et al., 2012). In addition, the researchers studied the CMTM7 in inhibiting function in vitro through the experiment of nude mouse. Research showed that the ectopic expression of CMTM7 suppressed the migration and growth of esophageal carcinoma cells effectively and induced the downregulation of BLNK in cancer cells, compared with non cancer tissues. In addition, Endogenous CMTM7 is partially localized with BLNK in A549 cells, which indicates that BLNK may also interact with human CMTM7 in non-leukemia cell line (Liu et al., 2015).

CMTM7 protein is mainly located in cytoplasm and cell membrane, expressed as a secreted protein in normal lung tissue, and the secretion of CMTM7 cannot be detected in some tumor vessels and bronchi, while it can be detected in some cells nucleuses. The localization of CMTM7 suggests the functional loss or arrhythmia of CMTM7 in non small cell lung cancer. Univariate analysis showed that the survival rate of the normal patients in expression of CMTM7 is higher than abnormal patients, and the up-regulation of CMTM7 expression in patients was the lowest survival rate and the survival rate was the lowest in patients with the up-regulation of CMTM7 expression (Liu et al., 2015). A recently research showed that CMTM7 knockdown may increase the oncogenicity and EGFR-AKT signaling by reducing Rab5 (a protein for early endosome fusion.) activation in human non-small cell lung cancer cells (Liu et al., 2015). In addition, some studies suggested that the potential function of CMTM7 is associated with heart failure mortality, like CMTM7 single nucleotide polymorphisms (SNP). Moreover CMTM7 and P16 have similar regulation system, and they all can be modified after translation, interaction and ultrastructure localization, to change the protein expression (Morrison et al., 2010; Harris et al., 2011; Zhang et al., 2014).

CMTM8

The difference of CMTM8 expression in esophageal cancer and cardiac cancer has statistical significance. The positive expression rate of CMTM8 is 100% in the

normal mucosa tissue adjacent to carcinoma, but 26.7% in the tumor tissue (Su et al., 2014). Chen et al. (2007) first used RT-PCR to research the expression of CMTM8 gene in human gastric cancer cell line BGC823, human leukemia cell line K562 and leukemia cell line HL-60.The result suggested that the expression of CMTM8 gene could be detected in the growth process of these three kinds of tumor cells.The transfection of CMTM8 could inhibit the proliferation of BGC823, K562 and HL-60.CMTM8 has a broad spectrum of immune cell chemotactic activation and can regulate cell proliferation, differentiation and apoptosis through some mechanisms, such as MAL domain inducing apoptosis, affecting the expression of EGFR, caspase and AIF and so on (Su et al., 2014).

The latest study indicated that T24 malignant cells proliferation, migration and invasion could be suppressed by the upregulation of CMTM8 on bladder cancer, with enhanced sensitivity to Epirubicin. CMTM8 could be identified as an significant prognostic indicator (Gao et al., 2015; Zhang et al., 2015).The loss of CMTM8 was thought to be associated with the clinical stage and the pathological grade of tumor (Gao et al., 2015).

Conclusion and Future Perspectives

The CMTM molecule is closely related with autoimmune diseases, hematological diseases and cardiovascular diseases. In recent years, a growing number of studies have found that the CMTM gene family plays an important role in the tumor's formation, development and metastasis. It has great clinical value for the diagnosis and treatment of tumor and immune related diseases. The study of CMTM has developed a new method and thought to explore the treatment of tumor. But at present, we don't know how the CMTM gene family influences the tumor's formation, development and metastasis, and we also don't clearly understand the specific molecular mechanism. In this sense, we need to do some further research.

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