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Review

Chaperone-mediated autophagy in cancer: Advances from bench to bedside

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Summary. Chaperone-mediated autophagy (CMA), a selective form of autophagy, where cellular proteins with KFERQ-like motif are targeted to the lysosome for degradation, is necessary to maintain cellular homeostasis. The role of CMA in neurodegenerative diseases has been extensively studied in the past decades, with defects in the pathway being strongly associated with disease. Recently, accumulating evidence has demonstrated a consistent increase in basal CMA activity in a wide array of cancer cell lines and human tumor biopsies, suggesting a potential link between CMA and cancer. On the other hand, an anti-oncogenic role for CMA under physiological conditions in non-transformed cells is also proposed despite the pro-tumorigenic function of CMA in cancer cells. The growing number of connections between CMA and cancers has generated interest in modulating CMA activity for therapeutic purposes. Here, we describe recent advances in the understanding of the molecular regulation of CMA, and discuss the evidence in support of the contribution of CMA dysfunction to cancers.

Key words: Chaperone-mediated autophagy, Cancer

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What is autophagy?

In 2016, Japanese scientist Yoshimori Ohsumi received the Nobel Prize in Medicine or Physiology for his discoveries in autophagy process. Recent progress in lysosome biology has paved the way to our current understanding of the autophagic mechanisms. Autophagy refers to the generic process of lysosomal degradation of intracellular components peculiar to eukaryotic cells (van Oosten-Hawle et al., 2013). There are two main ways to degrade intracellular molecules, one is ubiquitin-proteasome pathway and the other is autophagy-lysosome pathway. Accumulating evidence suggests that ubiquitin-proteasome systems are primarily responsible for the degradation of short-life proteins in cells, while the autophagy-lysosome pathway is primarily responsible for the degradation and utilization of long-life proteins and some organelles. According to the different ways in which intracellular substrates are transported to lysosomes, mammalian cell autophagy can be divided into three types, macro-autophagy, micro-autophagy and chaperone-mediated autophagy (Kaushik and Cuervo, 2012).

Type of autophagy

Macro-autophagy is a process in which the abnormal organelles or molecules in the cell are encased in a double-layer membrane to form an autophagosome, and then the autophagosome and lysosome fuse to form the autolysosome, and degrade its wrapped content, in order to achieve the metabolic needs of the cell itself and the renewal of certain organelles (Feng et al., 2014; Yu et

al., 2018). Micro-autophagy is mediated by lysosome activity by direct engulfment of the cytoplasmic cargo (Li et al., 2012). Chaperone-mediated autophagy (CMA) is a specific form of autophagy and is a highly selective method of autophagy (Cuervo and Dice, 1996). It can only selectively degrade certain proteins but not organelles, which can degrade proteins that contain specific amino acid sequences and can be identified and combined by heat shock cognate 70 (Hsc70) molecular chaperones (Majeski and Dice, 2004). The substrate protein is then transported directly into the lysosomes through a molecular companion and is degraded in the lysosomal body (Fig. 1).

What is CMA and how does it work?

Definition

CMA has a high degree of selectivity and uniqueness. It does not need to form a vesicle structure and the substrate protein directly goes through the lysosomal membrane (Mizushima et al., 2008; Kaushik and Cuervo, 2012). CMA has a basic expression in most cells, a basic method of inducing CMA is starvation. Unlike macro-autophagy (macro-autophagy can be activated by starvation treatment for 30 minutes and reach a peak by starving for 4-6 hours), CMA can be activated after 8-10 hours, and generally reaches a peak after 3 days (Cuervo et al., 1995; Massey et al., 2006).

CMA is mainly composed of three parts, lysosome associated membrane protein type 2A (LAMP2A), the molecular companion Hsc70 and the substrate protein molecules that can be identified by Hsc70 (Chiang et al., 1989). Molecular companion Hsc70 is a member of the heat shock protein family, capable of identifying protein molecules containing specific sequences, such as the five-peptide structure KFERQ-like motif contained in the substrate of the CMA (Dice, 1990). This particular

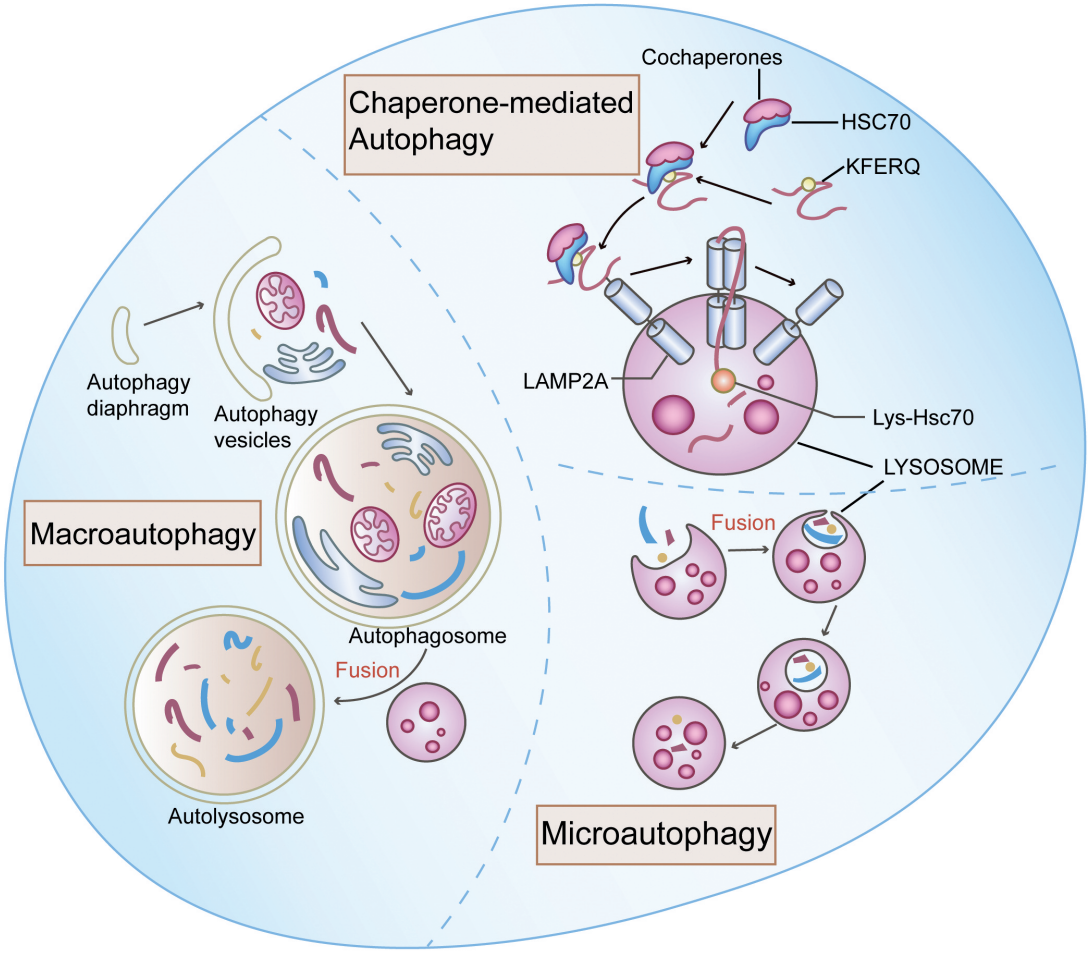

Fig. 1. Types of autophagy. Macroautophagy is a conserved catabolic process by which intracellular organelles or molecules are sequestered by double-membrane autophagosomes and then delivered to lysosomes for degradation and recycling in various physiological and pathological conditions. Microautophagy is mediated by direct lysosomal or vacuolar engulfment of the cytoplasmic cargo. Chaperone-mediated autophagy (CMA) is a specific form of autophagy and is a highly selective method of autophagy. It can only selectively degrade certain proteins but not organelles. The proteins that can be degraded contain specific amino acid sequences and can be identified by hsc70 molecular chaperone.

sequence can be combined with Hsc70 and then the substrate protein can be transported to the LAMP2A (Cuervo and Dice, 1996). Binding of the substrate protein to the cytosolic tail of LAMP2A drives LAMP2A multimerization to form a membrane translocation complex (Salvador et al., 2000; Bandyopadhyay et al., 2008). Then the substrate protein unfolds into the lysosomal body cavity, which is then degraded by the protease in the lysosomes, and the LAMP2A is separated to prepare for the combination and transshipment of the next substrate protein (Juste and Cuervo, 2019) (Fig. 2).

Cellular function of CMA

As a specific type of autophagy, the basic function of CMA is to provide amino acid recycling for cells with protein hydrolysate, especially in the state of starvation to provide energy for cells (Kaushik and Cuervo, 2018). The second important function of CMA is to provide quality control in cells (Arias and Cuervo, 2011).

In addition, depending on the protein substrate degraded, CMA has a modulatory role in a variety of cellular processes. This CMA-mediated selective remodeling of the proteome has recently demonstrated a role for CMA in modulation of carbohydrate and lipid metabolism, transcriptional programmes, immune responses and cell cycle control (Kaushik and Cuervo, 2018).

Dysregulation of CMA in diseases

Given the vital cellular function of CMA in maintaining cell hemostasis, dysfunction of CMA activity, such as reduced or enhanced, will contribute to diseases (Cuervo and Wong, 2014). Among them, neurodegenerative diseases, immune diseases and cancer have received the most attention from physicians. One of the most common features among the different neurodegenerative diseases is the aggregation of deleterious proteins or inclusions in neurons, including huntingtin protein in Huntington's disease, amyloid-β in Alzheimer's disease, and α-synuclein in Parkinson's disease. In those neurodegenerative diseases with impaired CMA activity, the substrate proteins cannot be degraded by CMA pathway, and these substrate proteins gather in the body, thus causing toxicity to the cells and pathological conditions (Campbell et al., 2018).

The role of CMA remains largely underappreciated in immune diseases, even though CMA has been claimed to play vital roles in major histocompatibility complex class II-mediated antigen processing and presentation. CMA is also identified to be an essential regulatory element of T-cell activation through the targeted degradation of negative regulators of TCR signaling, namely the ubiquitin ligase Itch and the calcineurin inhibitor RCAN1 (Valdor et al., 2014). Additionally, both LAMP2A and HSPA8 CMA markers were found to be over-expressed in lupus B cells from MRL/lpr mice which present many immune defects attributed to Fas deficiency (Page et al., 2011; Macri et al., 2015), providing a potential approach for lupus therapy.

Although there is accumulating evidence supporting the link between CMA and cancer, the precise role for CMA in cancer is still under debate. Findings from some studies are consistent with the idea that CMA facilitates the development and progression of certain cancers (Kon et al., 2011). But there is also increasing evidence demonstrating that CMA can inhibit cancer by specifically degrading oncogenic proteins. Given the scope of the journal and the research area we are focusing on, in this review, we will specifically focus on the recent findings linking CMA and cancers.

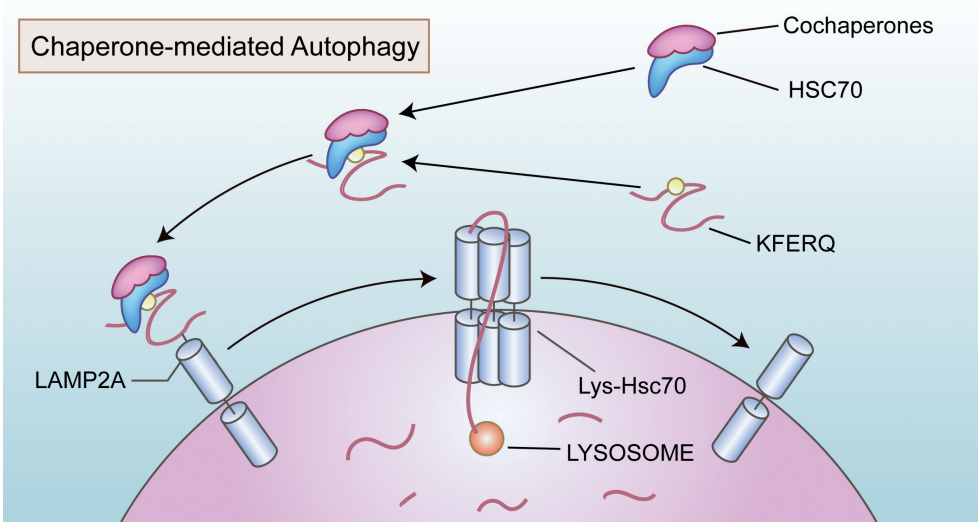

Fig. 2. Steps of CMA. The process of degradation of protein through CMA pathway is mainly done through four steps. As the first step, the substrate protein containing the KFERQ sequence binds to the molecular chaperone hsc70 to form a complex. At the second step, Hsc70/substrate complex interact with LAMP2A on the lysosomes membrane, which promotes lamp2A polymerization to form a transporter complex. The third step, upon unfolding, substrate proteins cross the lysosomal membrane assisted by a luminal chaperone. In the fourth step, the substrate protein in lysosomes is degraded under the action of lysosomes hydrolase.

Role of CMA in cancer

The strength of the CMA function is closely related to the occurrence and development of cancers. Clinically, it is found that the expression level of LAMP2A is significantly higher than that of normal tissue in many types of tumor tissues (Kon et al., 2011). Selective knocking out of LAMP2A at the gene level attenuated tumor growth (Massey et al., 2006). The current view is that increased CMA activity results in rapid tumor growth through modulating related protein levels. Firstly, the activity-enhanced CMA is able to selectively degrade basic proteins, providing energy for rapidly proliferating tumor cells and meeting the energy needs required for the proliferation and survival of tumor cells (Thorburn and Debnath, 2011). Secondly, highly active CMA can selectively degrade some of the speed-limiting enzymes in the process of energy metabolism, such as the process of glycolysis and key enzymes in the process of fat metabolism (Jacob et al., 2017). Existing studies have reported that CMA inhibits the occurrence of glycolysis during tumor growth (Tang et al., 2017). PKM2 is a speed limiting enzyme in the process of glycolysis, which can be degraded by CMA pathway. When CMA activity increases, PKM2 degradation increases, resulting in an increase in intermediate products such as 6-phosphate glucose, fructose-1,6-diphosphate and ATP levels in the process of glycolysis, which provides more energy and metabolic intermediate products for tumor cells, as well as raw materials for the synthesis of other biomolecules. Additionally, in the process of fat metabolism, such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as the substrate of the CMA, selective degradation can be achieved through the CMA, thus affecting the process of lipid metabolism. Thirdly, the rapid proliferation of tumor cells produces a lot of metabolic waste, as well as some oxidized and incorrectly folded proteins in the cells. Increased CMA activity can enhance the degradation of these proteins, thereby conferring stress tolerance and maintaining cellular survival (Kon and Cuervo, 2010; Kon et al., 2011).

However, in the early stages of the tumor, CMA plays a role in inhibiting the occurrence of the tumor (Cuervo and Wong, 2014; Wu et al., 2017). CMA is identified to play a tumor-suppressive role in non-tumorigenic cells by enhancing MYC's proteasomal degradation and thus abolishing its oncogenic activity by directly degrading carcinogenic proteins such as E3 ubiquitin-protein ligase MDM2 (Lu et al., 2010; Gomes et al., 2017). CMA may also protect against malignant transformation by assuring genome stability through its role in efficient DNA repair (Park et al., 2015). CMA has also been shown to participate in the protein degradation of another carcinogenic protein, the epidermal growth factor receptor pathway substrate 8 (eps8), which is associated with solid malignant tumors (Welsch et al., 2010). Although the complex role of CMA in cancer biology is yet to be explored, interventions aiming to restore CMA activity may have potential value in cancer prevention.

Interplay of CMA with different cancers

Altered CMA in cancers

Recently, accumulating evidences find that CMA activity is increased in most tumor tissues. But it is still unclear that what factors affect the activity of CMA during tumor development and progression. Recent studies have shown that certain biomolecules regulate the expression of LAMP2A, which affects the activity of the CMA. Sorting nexin 10 (SNX10), a cancer-suppressing gene, promotes the degradation of LAMP2A and inhibits the activity of CMA, thereby reducing p21 degradation (Zhang et al., 2018). In addition, phosphatase PHLPP1 can bind to the lysosome membrane and offset the inhibition of mTORC2 on CMA, thus activating CMA. The lysosome Akt is the target of the mTORC2/PHLPP1 kinase-phosphatase pair, regulating CMA activity by controlling the assembly and removal dynamics of the CMA translocation complex in the lysosome membrane (Arias et al., 2015). Furthermore, miR-320a is able to target CMA and suppress the activity of CMA (Li et al., 2014a).

Recent studies have found that CMA interplays with certain important tumor-related proteins. Mutant p53, which is known to actively contribute to tumor growth and metastasis, can be degraded through CMA (Vakifahmetoglu-Norberg et al., 2013). In addition, pro-apoptotic protein BBC3/PUMA (BCL2 binding component 3) is the substrate of CMA. Reduced CMA activity results in stabilization of BBC3, which in turn promotes cell apoptosis (Xie et al., 2015).

In general, in tumor tissues, the activity of CMA is modulated by the change of key proteins LAMP2A and Hsc70. Depending on substrate proteins, dysregulation of CMA may thus lead to different changes in cellular behavior.

CMA promotes cancers

In hepatocellular carcinoma (HCC) (Ding et al., 2016), it was found that LAMP2A was required for tumor growth and promoted tumor recurrence. Although a significantly lower level of LAMP2A expression was found in HCC cells and tissues compared with normal hepatic cells and para-tumor tissues, LAMP2A blockage significantly inhibited HCC cell viability under prolonged starvation. A significant correlation between LAMP2A expression and tumor size or cumulative recurrence was uncovered in HCC patients. Additionally, in clinical HCC specimens, CMA was found to compensate for impaired macro-autophagy in the cirrhotic liver to promote HCC survival (Chava et al., 2017). Liver cirrhosis is an independent risk factor for HCC. HCC was proved to undergo autophagy, switching from a protective state characterized by high macro-autophagy with low CMA to a HCC-promoting state

characterized by low macro-autophagy with high CMA when growing in the highly stressed cirrhotic microenvironment. Using persistently infected hepatitis C virus cell culture as the model system, it was found that CMA selectively targeted beclin1 degradation, leading to impaired autophagosome-endosome fusion, which then inhibited endocytosis and degradation of epidermal growth factor receptor (Aydin et al., 2018). More importantly, silencing Nrf2 and LAMP2A reduced cell viability, suggesting that the stress response activates CMA as a compensatory mechanism of cell survival. More recently, it was reported that HBV-associated exosomes modulate cell apoptosis and chemosensitivity via activating the CMA pathway (Liu et al., 2019). Therefore, a potential treatment strategy for hepatocellular carcinoma may be provided by targeting LAMP2A/CMA pathway in the CMA (Wu et al., 2017).

CMA can play a role in promoting the development of lung cancer (Cuervo and Wong, 2014). When the CMA is blocked, it can reduce the proliferation of human lung cancer cells and promote the death of human lung cancer cells. At the same time, when the CMA is blocked, it can cause metabolic changes in human lung cancer cells, such as response to stress and metabolic changes (Massey et al., 2006). In addition, when the CMA activity is reduced, the carcinogenicity of human lung cancer cells decreases. The metastasis ability of lung cancer cells decreased correspondingly after blocking LAMP2A. At the same time, it was found that the reduction of CMA expression could reduce the size of existing tumors. The study concluded that CMA could be a drug target for anticancer therapy, and that the CMA, which selectively blocks certain types of tumor cells, could be the basis for anticancer intervention.

In breast cancer, it was found that LAMP2A expression could promote the survival of tumor cells (Saha, 2012). Compared with normal tissues, the expression levels of CMA substrates, such as GAPDH, PKM, decreased in most breast cancer tissues. Reactive oxygen-mediated oxidative stress can damage substances in cells, such as DNA, proteins, and lipids. Protein carbon-based content (PCC) is widely used to measure the index of total cell protein oxidation. LAMP2A expression can reduce the content of PCC, thus protecting the survival of cells in the process of oxidative stress. In addition, when inhibiting the expression of LAMP2A, it can lead to the accumulation of GAPDH, the phosphorylation of AKT1, and the production of ROS, which can lead to apoptosis of breast cancer cells (Tokunaga et al., 2006; Liu et al., 2007). Chemotherapy drug polymorphism in the treatment of breast cancer will produce drug resistance over time. A recent study found that the expression of LAMP2A in breast cancer cells could enhance breast cancer cells' sensitivity to chemotherapy drugs. Therefore, locking the activity of the CMA may become a new target in the treatment of breast cancer.

In gastric cancer, it was found that the proliferation of gastric cancer cells was inhibited when LAMP2A was removed, together with changes in the expression of cell cycle-related proteins, including RND3 (Zhou et al., 2016). Further research found that RND3 interacted with CMA's key molecules, Hsc70 and LAMP2A, suggesting RND3 as a potential CMA substrate. More importantly, silencing RND3 partially reversed the cell growth inhibition effect induced by knocking out LAMP2A (Zhou et al., 2016). Therefore, the rapid proliferation of gastric cancer cells requires continuous degradation of RND3 through the CMA pathway.

CMA suppresses cancers

In pancreatic cancer, it was found that Vav1 can be degraded by CMA (Razidlo et al., 2013). As a member of the Vav family (Movilla and Bustelo, 1999), Vav1 plays an important role in the development and progression of many tumors, and thus has been considered as an oncogene (Katzav, 2007). The study found that Vav1 could be degraded by the CMA pathway by entering the lysosome through Hsc70 (Razidlo et al., 2013). Additionally, over-expression of Vav1 can promote tumor cell survival, proliferation, invasion and migration (Fernandez-Zapico et al., 2005; Razidlo et al., 2013). Therefore, by degrading Vav1, the growth of tumor cells can be inhibited.

In addition, there is another substrate that can be degraded by CMA in pancreatic cancer. It was found that epidermal growth factor receptor pathway substrate 8 (EPS8) could be degraded through CMA pathway, thus affecting the proliferation and growth of tumors (Welsch et al., 2010). As an epidermal growth factor receptor, the kinase activity regulates the activation of downstream substrates (Fazioli et al., 1993). There is growing evidence of increased EPS8 expression in many human solid tumors and malignant blood tumors, including the mouth, thyroid, esophagus, lungs, breast, pancreas, ovaries, cervical cancer and leukemia (Ding et al., 2013). In addition, the expression of EPS8 is closely related to the occurrence, proliferation, chemosensitivity and migration of tumors (Chen et al., 2008). Further studies have found that eps8 contains two KFERQ-like motifs that can be Hsc70 identified (Welsch et al., 2010). As the substrate protein of CMA, the degradation of EPS8 through CMA pathway in tumor cells may cause a biological characteristic change.

In ovarian cancer, the activation of the CMA is generally considered to promote the survival of tumor cells, but a novel mechanism has been found to eliminate cancer cells by inducing the activation of the CMA. Hexokinase2 (HK2) is an important enzyme in the process of glucose metabolism. Previous studies have found that the expression of HK2 is significantly higher in lung cancer, breast cancer, pancreatic cancer, hepatocellular carcinoma and ovarian cancer and is considered as a carcinogenic gene (Smith, 2000). In their study, HK2 was proved to be a CMA substrate. HK2 degradation through CMA pathway affected the process of glucose metabolism and further led to the death of

tumor cells (Xia et al., 2015). They revealed a novel mechanism by which excessive activation of CMA might be exploited pharmacologically to eliminate cancer cells. Their study thus delineates a novel pharmacological strategy to promote the degradation of HK2 in cancer cells.

In MDS/AML, it was found that AF1Q was able to enter into the lysosome for degradation through the CMA pathway (Li et al., 2014b). AF1Q was firstly found in acute myeloid leukemia (AML) (Tse et al., 1995), where elevated AF1Q expression was found. AF1Q, an oncogene, is not only associated with tumor invasive migration, but also promotes proliferation and chemotherapy resistance (Li et al., 2006; Co et al., 2010). By matching the protein sequence of AF1Q, six KFERQ-Like motifs were found, and the corresponding experimental methods proved that AF1Q could indeed be degraded by CMA (Li et al., 2014b). Additionally, recent studies have found that the key molecule of CMA--LAMP2A, is missing in Aza-resistant MDS cell lines and some AML cell lines (Dubois et al., 2019). The absence of LAMP2 is associated with Aza-resistance and hypersensitivity to lysosomes and autophagy inhibitors. Accordingly, cells that lack the expression of LAMP2 or have LAMP2 functional defects are found to be sensitive to Aza after being re-imported into LAMP2. Therefore, there is a strong correlation between LAMP2 deficiency and resistance to Aza and sensitivity to lysosome inhibitors. The authors propose that $CD34^{+}/LAMP2^{Low}$ patients during the course of treatment with Aza might benefit from a lysosome inhibitor already used in the clinic (Dubois et al., 2019).

Targeting CMA for cancer therapy

For different cancer types, the activity of CMA is beneficial or vicious dependent on specific substrate proteins (Fig. 3). In most tumors, enhanced CMA activity is required to promote tumor growth. Based on most current studies, there was a significant increase in the expression of LAMP2A in cancer cells. Repressive tumor growth, metastasis and drug resistance were observed when CMA was suppressed. As a result, blocking CMA could become a novel way to treat cancers.

At the same time, a portion of CMA substrate proteins may promote the growth of tumor cells, such as HK2 and mutant p53, which has been mentioned above. Therefore, for these tumor types closely related to HK2 or mutant p53, enhancing the CMA activity plays a vital role in fighting against cancer. Hence, selective activation of CMA can be treated as an alternative treatment strategy by removing pathological mutant proteins involved in human cancers.

In conclusion, CMA plays an important role in the development of cancer, and the biological characteristics of cancer cells are affected by selective degradation of specific substrate proteins. But for different types of tumors and different types of substrate proteins, CMA plays a different role in the anti-tumor process. On one hand, highly active CMA can promote the growth of tumor cells by degrading tumor suppressor proteins. On the other hand, highly active CMA can degrade the oncogenic proteins to inhibit tumor growth. Therefore, in the process of treating tumors, how to dialectically view the role played by CMA is very important.

Concluding remarks and future directions

In recent years, the identification of novel CMA substrates, a better understanding of molecular control of CMA pathway and the validation of relevance of CMA with cancers have expanded our understanding of the importance of CMA in multiple cellular functions. The

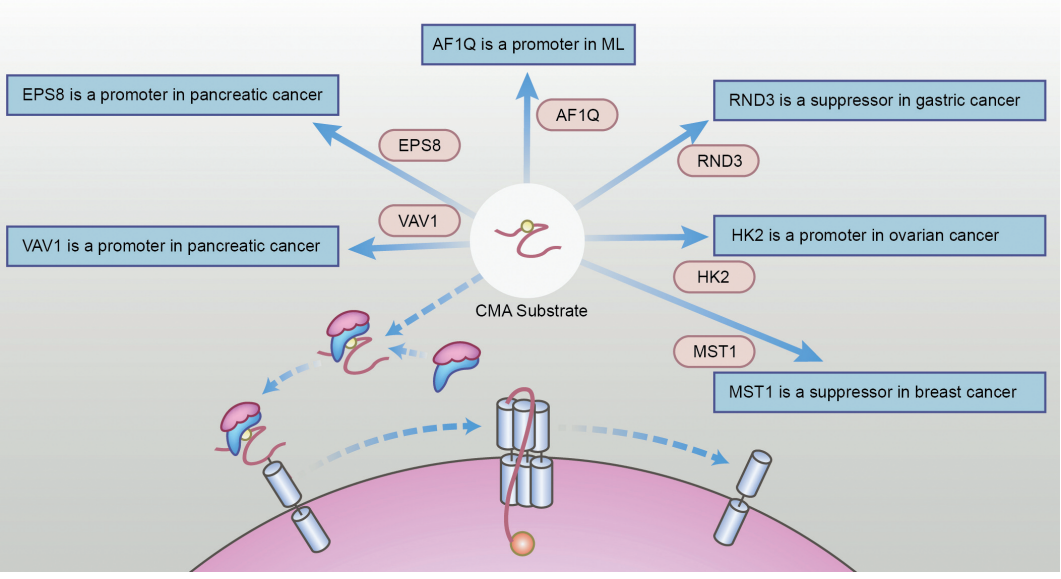

Fig. 3. Complex role of CMA in different cancers, Depending on the function of the CMA substrates, CMA was identified to promote or suppress tumor development and progression. Oncogenic proteins EPS8, Vav1, AF1Q and HK2 are CMA substrates in pancreatic cancer, AML and ovarian cancer respectively, while tumor suppressive proteins RND3 and MST1 are CMA suppressors in gastric and breast cancer respectively.

growing number of connections between CMA and cancers has generated great interest in modulating CMA activity for therapeutic purposes. Different substrate proteins in different cancers determine the different role of CMA in controlling cellular functions and cell fate. The complex relationship between CMA and cancer biology definitely warrants further studies to better understand the roles of CMA in cancer development and progression. Nevertheless, from the therapeutic point of view, manipulation of CMA is highly promising based on the fact that blockage of CMA in human tumor xenografts in mice through knock-down or knock-out of LAMP2A has proven effective in not only reducing tumor growth and metastasis but also inducing tumor shrinkage.

On the other hand, multiple questions also surround the dual role of CMA as an anti-oncogenic mechanism in normal cells but a pro-tumorigenic one in transformed cells. What are the molecular mechanisms that mediate the switch from low to high CMA during cell transformation and trans-differentiation? Does CMA confer chemo or radiotherapy resistance in some kinds of cancers? Are there any small molecule inhibitors or natural compounds that can specifically target the CMA pathway? How do oncogenic proteins protect themselves from being degraded by CMA in cancer cells? How does CMA communicate with other signaling pathways? The answers to those questions clearly require further in-depth studies. Clarifying these and other questions will further provide information of the possible therapeutic value of CMA modulation in cancers.

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