Principles, Processes and Modalities of Interaction Between Proto-Oncoprotein PIM-1 Kinase and Cell Division Cyclin CDC25A

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Abstract. All malignant tumors with aberrant cell differentiation and proliferation, unchecked growth, invasion, and metastasis are referred to as cancer. Its occurrence is a multi-step, complex process with many contributing components. Proto oncoprotein and The PIM kinase family is crucial to this procedure., and proto oncoprotein PIM-1 kinase is an important member of PIM family. The expression of proto oncoprotein PIM-1 is positively correlated with the status of lymphatic metastasis, and its overexpression could promote cell cycle and inhibit apoptosis. Cell division cycle 25 (CDC25) is a bispecific phosphatase from Schizosaccharomyces cerevisiae in 1986. CDC25 can activate CDK by catalyzing the dephosphorylation of inhibitory phosphorylation sites in the cell cycle dependent protein kinases (CDK) molecule, and regulate the response to DNA damage, thus promoting cell cycle operation. The interaction between the proto oncoprotein CDC25A in the above process is one of the keys to the current mechanism research with PIM-1 kinase. In this paper, PIM-1 and CDC25 are first introduced, respectively. Then, the interaction between PIM-1 and different substrates with a focus on CDC25A is presented. Finally, their role in tumor initiation and the application of CDC25A as a therapeutic target are highlighted. Therefore, this paper will discuss the interaction between CDC25A and PIM family.

Keywords: Cancer; Cyclin CDC25A; PIM Family Kinases; Apoptosis.

1. Introduction

1.1. Proto-oncoprotein PIM kinase

In humans, protein kinases regulate protein activity through phosphorylation and play a very important role in human's cell signaling. The largest family of proteins in the human genome is protein kinases. Up to now, more than 900 protein kinases have been found and confirmed. According to the amino acid residue species phosphorylated by its substrate protein, it can be divided into serine/threonine protein kinase, tyrosine protein kinase, histidine protein kinase, tryptophan protein kinase, and aspartine phthalase/glutamine phthaleinase.

Among them, Threonine kinase proto-oncoprotein PIM kinase is a member of the family of calcium/calcin regulating kinases. Among them, PIM-1 is the earliest discovered kinase, which plays a very important role in cell survival, value-added differentiation and apoptosis, as well as tumorigenesis and development.

Studies have shown that stimulation of cells by a variety of cytokines through their specific receptors can induce the expression of Pim-1mRNA and its proteins, and induce the binding of Pim-1 to a variety of STAT proteins, indicating that Pim-1 can be used as one of the important downstream elements of cell proliferation signaling pathways.

1.2. Cell division cyclin CDC25

By catalyzing the dephosphorylation of inhibitory phosphorylation sites in cell cycle-dependent protein kinase enzymes, a bispecific phosphatase known as CDC25 regulates the response to DNA damage and regulates the cell cycle. The key components of CDC25 are the three subtypes of CDC25A, CDC25B, and CDC25C, of which CDC25A is the "switch" protein that regulates the checkpoints of the G1/S and G2/M phases and is crucial for preserving the integrity of the cell division cycle as well as the stability of DNA replication. The gene encoding CDC25A is mapped to chromosome 3p21 and encodes tyrosine phosphatase. A regulatory domain at the N-terminus and a catalytic domain at the C-terminus make up the 524 amino acid residues that make up the CDC25A protein. The regulatory domain of CDC25A contains multiple phosphorylation sites, nuclear localization sequences, and nuclear output sequences; The phosphorylation site regulates the self-stability of CDC25A and its interaction with other proteins; Nuclear localization sequences and nuclear output sequences can modulate subcellular localization of CDC25A [1,2]. The catalytic domain of CDC25A contains an HCX5R motif, and the dipole moment of the adjacent α helix favors cysteine deprotonation and promotes the binding of CDC25A to substrates containing phosphorylated threonine and tyrosine groups [3]. In addition, the activity of CDC25A is regulated by multiple levels, including transcription level, translation level, and post-translation modification level [4]. CDC25C is a cell division cycle protein that plays an important regulatory role in eukaryotic mitosis and is one of the key factors for cells to enter the M phase. CDK1/cyclin B activated by CDC25B activates CDC25C and regulates mitosis through a positive feedback mechanism.

1.2.1 Oncogenic effects of cell division cyclin CDC25A

CDC25A promotes cell entry into the S phase through dephosphorylation of cyclin-dependent kinase 2-cyclin E complex (CDK2-cyclin E) and CDK2-cyclin A during the G1/S transition phase. Therefore, the high expression of CDC25A can accelerate the transformation of G1/S phase, and down-regulation of its expression will lead to S phase blockage. During the G2 and GM transition phase, CDC25A and CDC25B can synergistically promote the polymerization of chromatin in mitosis. If the expression level is down-regulated, it will lead to a slowdown in entering the G2 stage, while overexpression will lead to disordered mitosis. CDC25A as a key regulator of the G1 and GS phase and G2/M phase of the cell cycle, it will encourage the crossover during the G1/S phase and the G2/M phase checkpoint, leading to unchecked cell proliferation and the development of cancer.

1.2.2 CDC25A inhibits the process of apoptosis

CDC25A can enhance the stability of FOXO1 by dephosphorylating CDK2, while FOXO1 promotes tumor cell metastasis by directly regulating the transcriptional activity of matrix metalloproteinase (MMP1), a tumor metastasis-related factor.

1.3. The role of PIM-1 and CDC25A in tumors

Since DNA damage checkpoints are an important mechanism for inhibiting tumorigenesis, when DNA is damaged, cell cycle checkpoints are immediately activated to halt cell cycle progression. At present, it is believed that abnormal sensors, transducers and effectors in the DNA damage checkpoint pathway will lead to a decrease in gene stability, which is a necessary factor for the emergence and development of malignant tumors. DNA damage signaling activates CHK1 and/or CHK2, CHK1 and CHK2 can phosphorylate a series of tryptophan on CDC25A, resulting in hydrolytic destruction or loss of phosphatase activity, causing the cell cycle to continue when DNA damage occurs, apoptosis to be inhibited, and eventually lead to the proliferation of genetically defective cells and the formation of tumors.

In terms of CDC25A research, at present, Cdc25A has been unanimously identified by scholars as having oncogene function, and there is overexpression in many tumor cells. Yamashita analyzed the expression level of Cdc25A in human glioma tissue and human brain tissue, and the results showed that the expression level of Cdc25A in glioma tissue was higher than that of normal brain tissue [5].

In terms of the interaction between PIM1 and cdc25A, Cohenpointed out that a variety of signaling stimuli (cytokines, hypoxia, toxins, etc.) can induce PIM1 expression, causing phosphorylation of its downstream proteins and participating in many biological processes [6]. Previous studies by Feldman and Qian have shown that PIM1 plays an important regulatory role in biological processes such as cell proliferation, invasion, metastasis, apoptosis, cell cycle, chemotherapy resistance and other biological processes by phosphorylating genes and a series of substrate molecules involved in cell proliferation, such as CDC25A/C [7,8].

Studies have shown that various cyclin-dependent kinases (CDK) and cyclin-dependent kinase inhibitors (CKI) are substrates for PIM-1 kinase activity, and that cyclin, cyclin-dependent kinase (CDK), and cyclin-dependent kinase inhibitor play a major role in controlling the cell cycle (CKI). PIM-1 can directly bind to the cell division cyclin cdc25A, a positive-specific G1 phase regulator that promotes the transformation of the cell cycle from G1 phase to S phase. PIM-1 kinase participates in the cell cycle by dividing cyclin and significantly enhancing its phosphatase activity. Bachmann et al. confirmed that both C-TAK1 and Cdc25A are substrates of PIM-1, which play an important role in the transition of cells from the G2 phase to the mitotic phase [9]. On the one hand, PIM-1 can phosphorylate C-TAK1 to lose the ability to inactivate Cdc25A, and on the other hand, it can activate Cdc25A through phosphorylation, thereby greatly accelerating the process of cells entering MSS from G2 phase.

2. Substrates of PIM-1

2.1. CDC25A/C

The cell division cycle gene CDC25 was isolated from fission yeast in 1986 and is named for its ability to initiate mitosis, and is a member of the cell cycle regulators, whose expression product is a class of protein phosphatases with a relative molecular mass of 65×103 and dual tyrosine/threonine properties that activate cyclindependent protein kinase (CDK) by dephosphorylation, thereby driving the cell cycle. The three identified CDC25 phosphatases in mammals are CDC25A, CDC25B and CDC25C, of which CDC25A is the most important member that functions throughout all phases of the cell cycle and plays an important role in mitosis and physiological activities. It is also a regulator of apoptosis and a major node in the DNA damage checkpoint pathway to maintain gene stability. The uncontrolled expression level can lead to cell cycle disruption, reduce the stability of DNA, and promote the proliferation of malignant tumor cells.

The gene encoding CDC25A is localized on chromosome 3p21 and encodes a tyrosine phosphatase. CDC25A protein consists of 524 amino acid residues and contains an N-terminal regulatory domain and a C-terminal catalytic domain. CDC25A is unanimously considered to function as an oncogene and is overexpressed in many tumor cells, involved in the process of tumorigenesis and progression, and associated with tumor malignancy and prognosis.

CDC25C is a cell division cycle protein that plays an important regulatory role in eukaryotic mitosis and is one of the key factors for cell entry into M phase. Activated by CDC25B, CDK1/cyclin B activates CDC25C and regulates mitosis through a positive feedback mechanism.

2.2. Notch

Notch is a family of genes encoding transmembrane receptors in mammalian cells that are involved in growth regulation, apoptosis and differentiation of embryonic development and normal cells. The Notch signaling pathway is highly conserved in evolution, suggesting a critically important biological function. Notch has been identified in drosophila, and there are four members of Notch1-4 in mammals. Notch has important functions in animal neurodevelopment and may have different regulatory effects on neural stem cells at different stages of neurodevelopment. It has been shown that during the proliferation of neural stem cells, the Notch signaling pathway can maintain their stem cell properties, and during differentiation, Notch activity can inhibit the differentiation of neural

precursor cells into neurons and oligodendrocytes and promote their differentiation into astrocytes. However, blocking the notch signaling pathway did not have a significant effect on the cell viability.

2.3. Myc

Up to 15% of human genes are regulated by the oncogenic transcription factor c-Myc (also known as Myc), which participates in the cell cycle and important processes such as cell growth, apoptosis, differentiation, and metabolism. Myc directly binds to the E-box in the promoter region of genes to control the expression of a variety of genes. Growth factor-dependent signaling closely regulates its expression levels in normal cells, but it is unregulated and increased in tumor cells by a number of methods.

The c-Myc transcription factor is encoded by the MYC gene, which dimerizes with another helix-loop-helix leucine zipper protein, MAX, to bind DNA and regulate gene expression. The c-Myc protein is disordered in the absence of dimer formation, but when bound to the MAX protein can form a heterodimer with a helical structure and bind to the gene initiation E-box region, thereby activating or repressing downstream genes. MYC has oncogenic potential, and under normal conditions, the MYC gene is tightly regulated during transcription, post-transcriptionally and post-translationally, and once it is activated through chromosomal translocations Under normal conditions, MYC genes are tightly regulated during transcription, post-transcription and post-translation.

According to the "Warburg effect", tumor cells take up large amounts of glucose for aerobic glycolysis to meet the high demand for ATP for rapid cell growth and invasion and metastasis. c-Myc can assist cells to take up glucose for aerobic glycolysis by increasing the expression of glucose transporter (GLUT) or upregulating key enzymes in the glycolytic pathway to promote epithelial cell mesenchymalization and enhance cell migration and invasion, c-Myc can assist cellular uptake of glucose for aerobic glycolysis, promote epithelial cell mesenchymalization, and enhance cell migration and invasion by increasing the expression of GLUT or upregulating key enzymes in the glycolytic pathway. The overexpression of c-Myc promotes LDHA expression, maintains glycolytic flux, and produces excess lactate leading to extracellular matrix acidification, reducing the ability of T cells to export lactate, thereby decreasing T cell activity and playing a positive role in promoting tumor immune escape. In addition, c-Myc can also enhance nucleotide biosynthesis in tumor cells.

3. Interaction between CDC25A and PIM

The proto-oncoprotein PIM kinase is a threonine kinase that is belong to the calcium/calmodulin-regulated kinase family. Among them, PIM-1 is the first kinase identified, which has an important role in cell survival value-added differentiation and apoptosis as well as tumorigenesis and development.

In both in vitro and in vivo settings, Pim-1 physically interacts with Cdc25A, after that Pim-1 phosphorylates it. Additionally, Cdc25A's phosphatase activity is elevated as a result of Pim-1-mediated phosphorylation. In regards to the interaction between PIM1 and cdc25A, Cohen noted that a variety of signaling stimuli (cytokines, hypoxia, toxins, etc.) can induce PIM1 expression and cause phosphorylation of its downstream proteins to participate in many biological processes [10]. Feldman and Qian have shown that PIM1 plays an important regulatory role in cell proliferation, invasion, metastasis, apoptosis, cell cycle, chemoresistance and other biological processes by phosphorylating genes involved in cell proliferation such as cdc25A/C, and a series of substrate molecules [4,7].

A number of cell cycle protein-dependent kinases (CDK) and cell cycle protein-dependent kinase inhibitors (CKI) are substrates for the action of PIM-1 kinase, and the operation of cell cycle is mainly regulated by cell cycle proteins, CDK and CKI, etc. The PIM-1 kinase has been involved in the cell cycle through the cell division cyclin and significantly enhances its phosphatase activity. On the one hand, PIM-1 phosphorylates C-TAK1 to deprive it of its ability to inactivate Cdc25A, and on the other hand, it activates Cdc25A by phosphorylation, which greatly accelerates the cellular transition from G2 phase to MSS.

4. Tumor/carcinogenic effects

4.1. Carcinogenic effects

4.1.1 CDC25A exerts its oncogenic ability through its action on the cell cycle.

During G1/S transition, it promotes cells to enter S phase through dephosphorylation of cyclin-depend on kinase 2-cyclin E (CDK2-cyclin E) and CDK2-cyclin A. Therefore, high expression of CDC25A accelerates the G1/S phase transition, while downregulation of its expression leads to S phase arrest. During G2/M transition, CDC25A promotes chromatin polymerization in mitosis with the synergistic effect of CDC25B. Down-regulation of CDC25A expression results in slower entry into G2 phase, while overexpression results in disordered mitosis.

An increase in CDC25A expression increases the transition between the G1/S and G2/M checkpoints, which results in unchecked cell proliferation and malignant transformation. CDC25A is a key regulator of the G1/S and G2/M stages of the cell cycle.

4.1.2 Inhibition of apoptosis by cdc25A

Aberrantly expressed CDC25A can prevent apoptosis and induce malignancy by affecting the bioactive abundance or localization of apoptosis-related factors. A key player in the control of apoptosis is the mitogen-activated protein kinase kinase (MAP3K) family member known as apoptosis signal-regulating kinase 1 (ASK1). CDC25A can inhibit ASK1 kinase activity by binding to the ASK1 kinase activation binding region via the carboxyl terminus, thereby blocking apoptosis.

4.1.3 CDC25A and DNA damage response

The DNA damage checkpoint is an important mechanism for tumor suppression. When DNA is damaged, the cell cycle detection site is immediately activated and the cell cycle process is suspended. DNA damage signals activate CHK1 and/or CHK2, which phosphorylate a series of tryptophan on CDC25A, leading to its hydrolytic destruction or loss of phosphatase activity, resulting in cell cycle arrest. The cell cycle continues during DNA damage and apoptosis is inhibited, leading to the proliferation of genetically defective cells and tumorigenesis.

4.1.4 CDC25A promotes tumor metastasis

CDC25A enhances the stability of FOXO1 by dephosphorylating CDK2, and FOXO1 promotes tumor metastasis by directly regulating the transcriptional activity of the tumor metastasis-associated factor matrix metalloproteinase 1 (MMP1).

4.2. Application as therapeutic target in cancer therapy

As a crucial node in the cell cycle checkpoint network, many abnormally expressed oncogenes can play a valuable role by increasing the expression level of CDC25A, and CDC25A and its abnormal expression can also affect other tumor-related factors in the body. In terms of treatment, Naderi et al. found that co-inhibition of CDC25A and androgen receptor (AR) in estrogen receptor (ER)-negative molecular apocrine breast cancer could be used to reduce cell mutation and growth more effectively than a single approach. Five genes, including CDC25A, were found to be markers for the efficacy of radiotherapy in colorectal cancer. Removal of CDC25A from the skin of mice under UV irradiation accelerated the elimination of DNA damage, but did not yet fundamentally alter the cell cycle process and tumorigenesis. These studies suggest that inhibition of abnormally elevated CDC25A protein expression levels have significant therapeutic implications for a variety of cancers.

Theoretically, the levels of intracellular phosphatases are affected at the levels of transcription, translation, post-translational modification, degradation, protein-protein interactions and subcellular localization, making each of these levels a target for the development of small molecule therapeutics. Gubanova et al. demonstrated that a novel tumor suppressor, SMG-1, could inhibit CDK2-driven tumor cell proliferation by reducing the stability of CDC25A. Recently, some mi RNAs are found to inhibit CDC25A expression. found that miR-483-3p could induce CDC25A silencing by binding to

the UPR transcription site of CDC25A, inactivating CDK4/6 and arresting the cells in G1 phase, and further demonstrated its inhibitory effect on the proliferation of squamous cell carcinoma cells.

Among the natural drugs used in traditional medicine, many components have been shown to directly inhibit the expression of CDC25A and thus produce antitumor effects. Triptolid (TPL), extracted from the Chinese herb Radix Rehmanniae, can induce apoptosis in human melanoma A375.S2 cells by inhibiting the expression of cyclin E and CDC25A and blocking them in the S phase. A plant called Critonia morifolia (Asteraceae) can treat various inflammatory diseases, and Unger et al. found that polar extracts from this plant can induce apoptosis by inhibiting a series of cell cycle-related factors, such as the transposable gene products c-myc, CDC25A and CDC25C. 3, 3'-Diindolylmethane is a plant from Brassica juncea. 3,3'-diindolylmethane is a drug with anticancer potential obtained from Brassica napus. Through many signaling pathways, it can stop human breast cancer cells from proliferating.These include phosphorylation of the Ser124 site on CDC25A, which induces the degradation of CDC25A.

Inhibition of the catalytic domain of CDC25 phosphatase is the target of choice for synthetic drugs. In recent years, the effective inhibitors of CDC25S are natural extracts or synthetic products based on strong acidic, highly electrophilic or quinone-like structures, which are very effective inhibitors, but they are not specific for CDC25A/B/C and generate reactive oxygen species during the inhibition process, which can damage the cell function. In this regard, Tsuchiya et al. identified the o-hydroxybenzyl derivative RE44, which specifically inhibits CDC25A/B. found that Arylstibonic acid selectively inhibits CDC25A and CDC25B. By expressing and purifying the fusion protein of CDC25A/B catalytic region with glutathione-S-transferase (GST). Researchers established a high-efficiency high-throughput screening system for target enzyme inhibitors, which laid the foundation for further development of VK3 derivatives as novel targeted anticancer drugs. Simvastatin has also been shown to act on the CHK1-CDC25A-cyclin A/CDK2 pathway to induce S-phase arrest in a variety of multiple myeloma cells.

5. Conclusion

A variety of signal stimuli (cytokines, hypoxia, toxins, etc.) induce PIM1 expression, causing phosphorylation of its downstream proteins, including PIM-1 phosphorylation modification of cdc25A, thereby enhancing the activity of CDC-25A. The interaction of PIM-1 and CDC25A makes it more carcinogenic in vivo. On the other hand, CDC25A can be applied as a therapeutic target. Among natural and synthetic drugs, many of them play a role in the treatment of tumors through their interaction with CDC25A.

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