Research Progress on the Function of Chk2 Protein

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Abstract: Cell cycle checkpoint kinase 2 (Chk2) is a cell cycle monitoring kinase that exists widely in mammals. CHK2 is activated by phosphorylation and has a series of reactions with a variety of downstream proteins, which are active in various cellular reactions of organisms, such as DNA repair, cell cycle regulation and apoptosis. In this review, various biological functions of CHK2 are reviewed, with emphasis on cell cycle control, apoptosis, attenuated angiogenic mimicvm formation and autophagy in tumor cells with p53 mutation. The summary of this review will help us to have a clearer and more comprehensive understanding of the biological functions of CHK2, and provide more effective treatment options for some diseases through the study of CHK2.

***Keywords*:** CHK2, DDR, autophagy, cell cycle

1. Protein structure characteristics of CHK2

Chk2 is located on chromosome 22q12.1, which contains 14 exons and its cDNA is 1731bp and the Chk2 mRNA is widely expressed in normal tissues. It can be found in all stage of cell cycle.[1] The 60kDA coding protein is consisted of 543 residues and including three different domains: a region rich in serine-glutamine/threonine-glutamine amino acid pairs which is called SQ/TQ cluster (SCD). Forkhead-associated (FHA)domains which is between residues 112 and 175. FHA domain is about 140 AA length and a pThr-binding module in eukaryotic proteins e.g. kinase, transcription factors, phosphatase and etc., it also can interaction with phosphorylated SCD.[2] Kinase domain (KD) across 220-486 residues at C-terminal. To maintain the kinase domain(KD) activity, the three domains on Chk2 are indispensable.[3]

2. Traditional biological functions of CHK2

Chk2 is the key Effector protein kinase in cell cycle, when DNA damage or replication block, the genomes' stability relies on DNA repair system and Checkpoint regulation to Coordinate. Chk2 was structurally expressed throughout the cell cycle and existed as inactive monomer in cells without DNA damage. When DNA double-strand break damage occurs, phosphatidylinositol 3 kinase family checkpoint protein kinase ataxic Telang dilatation mutation (ATM) phosphorylates Chk2[4], which as an important signal transduction protein in DNA damage repair pathway, it plays a role as signal distributor and split the check point signal into downstream target proteins to regulate cell cycle process through phosphate and active the p53, BRCA1, E2F-1, Cdc25A and other downstream target cell which can perform DNA repair, cell cycle regulation and apoptosis. Finally, the cell uses various pathway to active the G1/S and(or) G2/M checkpoint to block the cell cycle and active the repair gene to promote cells to repair damage.[5]

2.1 Chk2 participates in apoptosis

P53 is a co-regulator of both endogenous and exogenous apoptosis and is associated with checkpoint activation or apoptosis during DNA damage.[6] The p53 is the most common target gene in malignant tumor development of human and over half human tumors occur p53 mutation.[7] When DNA damage occurs, ATM and Chk2 phosphorylate p53 to induce its stable expression in the nucleus and induce apoptosis.[8] BRCA1 widely expressed in various tissues especially in testis and thymus. Recently research find that BRCA1 can induce cell apoptosis, high expression of BRCA1 can increase Gadd45 expression, which can active NK/SAPK upstream regulator MTK1/MEK4 and induce JNK/SAPK dependent cell apoptosis. [9] CHK2 phosphorylates E2F-1 ser364 in response to DNA damage induced by the agent etoposide, which regulates stability and transcriptional activity of E2F-1.[10] Given that e2F-1-deficient thymocytes have been observed to be resistant to etoposide-induced apoptosis [11] and that overexpression of E2F-1 can induce cell death, the regulation of CHK2 activity on E2F-1 represents that CHK2 can regulate DNA damage-induced apoptosis. CDC25a is also a downstream protein.

2.2 Chk2 is involved in regulating cell cycle

When the DNA double strand breaks under the action of external factors such as ultraviolet light, CHK2 is activated and

interacts with Cdc25 to regulate the cell cycle, and causes the cell cycle to be blocked at certain stages to repair the damaged DNA And this process requires the participation of ATM.[12] When cellular DNA is damaged, The DNA damage signal will stimulate the phosphorylation of CHK2 with the participation of ATM and finally change the Conformation of cyclin25A, leading to its inactivation which makes CDK lose its ability to bind to it and cannot be activated, thus causing the cell cycle to be suspended.[13] It directly affects the CDC2 /cyclinB1 complex and makes it lose its original function, which leads to cell stagnation in the G2/M phase.[14] Phosphorylation of Cdc25C at S287, leading to binding of 14-3-3 protein, leads to arrest in the G2 phase of the cell cycle.[15] Chk2 ensures the normal operation of each cell cycle by regulating the cell cycle, and its blocking effect on the cell cycle also provides an opportunity for cells to repair DNA damage.[16]

2.3 Chk2 is involved in cell division

Chk2 is also active in mitosis and indirectly affects mitotic chromosome separation by regulating normal physiological functions of centrosomes. Chk2 has been shown to react with a substance called Myosin Phosphatase Targeting Subunit 1 (MYPT1) at S507, this change prevented phosphorylation of S473 of MYPT1 during normal mitosis. This causes gamma-tubulin to fail to recruit, disrupting centrosome assembly process.[17] In addition, Chk2 also maintains correct assembly of mitotic spindles by phosphorylation of BRCA1.[18] This tells us that CHK2's effect on cell division is important, a process that is critical for maintaining genetic stability in organisms.

2.4 Chk2 is involved in the formation of attenuated vasculogenic mimicry VM in tumor cells with p53 mutation

Chk2 is involved in attenuating the formation of angiogenic mimicry VM in tumor cells with p53 mutation. Experiments have shown that the level of VE-cadherin in cells with high phosphorylation of Chk2 T68 is significantly higher than that in cells with low phosphorylation of Chk2 T68 [19], suggesting that Chk2 activation plays a role in reducing VM formation. PKM2 is greatly expressed in tumor cells, replacing the original pyruvate kinase PKM1, etc., which leads to the improvement of glucose uptake, and the way of energy acquisition of cells also changes from oxidative phosphorylation to glycolysis[20]. Chk2 interacts and phosphorylates with serine residues at the S100 site of PKM2 to promote its transport from the nucleus to the cytoplasm. Once PKM2 leaves the nucleus, it cannot promote the transcription of target genes, such as the targeted expression of PKM2 and VEGF-A[21]. This process interferes with glucose flux and affects the PKM2-controlled citric acid cycle, glycolysis, and pentose phosphate pathways[22], ultimately blocking the formation of angiogenic mimicry VM and thereby inhibiting the growth of cancer cells.

2.5 Chk2 participates in autophagy

When DNA damage in a cell accumulates beyond repair, the cell will end up autophagy. It was found that CHK2 was also involved in autophagy in the absence of DNA damage but accompanied by energy deprivation and metabolic fluctuations. Chk2 mainly binds to Beclin 1 to phosphorylate Ser91 and Ser93 of Beclin1 respectively, thereby disrupting the formation of the Beclin 1-Bcl-2 autophagy regulatory complex in a ROS-dependent manner, leading to the autophagy process of cells[23], which is conducive to the maintenance of cellular homeostasis.

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