



University of Mazandaran

Journal of Genetic Resources
J Genet Resour 2015;1(1): 31-34
<http://sc.journals.umz.ac.ir>
doi: 10.22080/jgr.2015.1115



Iranian Biology Society

The Role of *chk2* in Response to DNA Damage in Cancer Cells

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Received: 21 December 2013 Accepted: 05 December 2014

Abstract

Accumulation of gene changes and chromosomal instability in response to cellular DNA damage lead to cancer. DNA damage induces cell cycle checkpoints pathways. Checkpoints regulate DNA replication and cell cycle progression, apoptosis, and chromatin restructuring. Checkpoint kinase 2 (*chk2*) is activated in response to DNA lesions. ATM phosphorylates *chk2*. The activated *Chk2* kinase can phosphorylate the substrates, including the *Brca1*, *E2F-1*, *PML*, *p53*, and *Cdc25* phosphatases, which has been associated with DNA repair, cell cycle arrest or induction of apoptosis. However, *Chk2* is a tumor suppressor gene that maintains genomic integrity and it has been suggested as an anticancer therapy target.

Key words: *Chk2*, DNA damage, Cancer cell, Therapy, apoptosis, checkpoint

Introduction

Genomic instability is a main characteristic of cancer cells as it increases mutation and contribute to development of the malignant state. There are various types of genomic instability including, microsatellite instability due to mismatch repair deficiency, CpG island methylator phenotype (CIMP) and chromosomal instability that occur in cancer cells (Abdel-Rahman, 2008) (Saldivar *et al.*, 2012). During cancer development, genomic instability spreads due to a combination of factors including oxidative stress, telomere shortening and defective DNA damage response. The checkpoints are activated by incomplete DNA replication or DNA damage and cause cell cycle arrest as well as inhibition of DNA replication after DNA damage. DNA damage checkpoints participate in carcinogenesis and cancer therapy (Carrassa *et al.*, 2004). Tumor cells have checkpoint defects in G1 and G2 checkpoint proteins. G2 checkpoints have a key role after DNA damage. *Chk1* and *chk2* kinase have been identified as key regulators of the G2 checkpoint after DNA damage.

These checkpoints delay transitions from G1 to S, G2 to M or inhibit DNA replication during S-phase. *Chk1* and *Chk2* phosphorylate a number of

proteins that result DNA repair, apoptosis, G1 arrest, G2/M arrest and S-phase arrest. These delays and cell cycle arrest allow time for repair processes or halt cell cycle progression until completion of a critical cell cycle event (Cliby *et al.*, 1998). *Chk2* is expressed throughout the cell cycle and appears to be inactive in the absence of DNA damage, it is activated by ATM (Ataxia telangiectasia mutated) in response to double-strand DNA breaks (Bartek, 2003). The *Chk2*-deficient mice do not show a tumor phenotype until exposure to carcinogens. The *Chk2*^{-/-} mice have increased resistance to ionizing radiation, cellular defects in *p53* function, some checkpoint responses, and apoptosis (Bartek, 2003). Here, we review the function and role of *chk2* in response to DNA damage in cancer cells and discuss *Chk2* as a target therapy in cancer treatment.

DNA damage induced *chk2* kinase

Environmental factors including ultraviolet light (UV), ionizing radiation and oxidative stress cause mainly single- and double strand breaks. These lesions must be repaired. Checkpoints in the cell cycle are activated in response to DNA damage and prevent progress of the cell cycle

thus, DNA lesions repair, senescent or cell death programmed is activated. These responses to DNA damage are also induced in cancer therapy by chemotherapy drugs and radiotherapy (Perona *et al.*, 2008). After DNA damage, chk2 is activated by ATM and phosphorylated on Thr68 (Perona *et al.*, 2008). On the other hand; Chk2 is activated as a tumor suppressor and enhance apoptosis by stabilizing p53 (Huang *et al.*, 2008; Jobson *et al.*, 2009).

Chk2 is a key protein in apoptosis and DNA repair

DNA damage such as double-strand breaks is received by sensors such as HUS7, RAD1, RAD9 and RAD17 then relays to ATR and ATM, which phosphorylate and activate the checkpoint kinase-1,2 (chk1,chk2), which halt the cell cycle (Castedo *et al.*, 2004). Checkpoint kinases (chk1, chk2) suppress the activation of the Cdk1/cyclin B complex which required for the entry into mitosis (Castedo *et al.*, 2004).

Chk2 phosphorylate several substrates, including p53, Cdc25C, Cdc25A, the promyelocytic leukemia protein (PML), E2F-1 and Brca1 which mediate apoptosis, cell cycle arrest and DNA repair. Chk2 phosphorylates the dual-specificity phosphatase Cdc25C which promotes its binding to the 14-3-3 protein and results in its sequestration into the cytoplasm. Because Cdc25C activate CDK1 at the G2/M transition in the nucleus, this induces cell cycle arrest in the G2 phase and prevents cells from entering mitosis in the presence of DNA damage. Chk2 phosphorylates Cdc25A which promotes its binding to the SCF ^{β -TrCP} -ubiquitin ligase complex and result Cdc25A degradation also prevent the activation of CDK2 at the G1/S transition (Huang *et al.*, 2008; Stolz *et al.*, 2011). Additionally, P53 is a key target for Chk2, whereas chk2 phosphorylate and activate p53. P53 activates p21, which activates G1/S checkpoint by inhibiting two G1/S cyclin-dependent kinases (CDK2 and CDK2). In the S phase Cdc24A are phosphorylated by Chk1 and Chk2, thereby occur DNA repair (Stolz *et al.*, 2011). Otherwise, Chk2 phosphorylate FOXM1, which activates the expression of BRCA1. The function of BRCA1 associates with DNA mismatch repair proteins. It suggests a possible involvement chk2 and Brca1 in mismatch DNA repair. When DNA damage cannot be repaired, the damaged cell induces

apoptosis, which can be regulated by the Chk2 kinase. In fact, Chk2 induce p53-dependent apoptosis or p53-independent apoptosis by phosphorylating the transcription factor E2F-1 (Zhang *et al.*, 2004; Huang *et al.*, 2008; Stolz *et al.*, 2011). The E2F-1 is induced by cellular stress and regulated during cell cycle by chk2. Chk2 mutation prevents induction of E2F-1 dependent apoptosis (Stevens *et al.*, 2003). Chk2 can also phosphorylate the tumor suppressor PML which promotes pro-apoptotic pathway in a p53-independent manner. Chk2 phosphorylation lead to p53 activation, then p53 activates p21, contributing to G2/M inhibition (Perona *et al.*, 2008). p53 regulate target genes, such as Bax, Noxa and Puma that induced apoptosis in response to DNA damage (Zhang *et al.*, 2004).

Chk2 alter in cancer

Chk2 mutation has been identified in several cancers including breast, colon, prostate, and lung carcinomas. Loss of the chk2 locus on chromosome 22q13 has been reported in breast, colorectal, ovarian, and brain tumors. Germ line mutations of chk2 have been found in Li-Fraumeni syndrome. Concomitant mutations in chk2 and TP53 have been observed in colon and breast cancer (Perona, *et al.*, 2008; Stolz *et al.*, 2011). The lack of FHIT Fragile Histidin Triad and disruption of the chk2 is detected in oral squamous carcinoma. FHIT is tumor suppressor gene that regulates chk2 phosphorylation in DNA damage pathway (Yutori *et al.*, 2008). Thus chk2 activation can be regulated by tumor suppressor genes. Chk2 inhibition enhance the level of mitosis in combination with either doxorubicin or cisplatin. Chk2-null mice increase survival when exposed to ionizing radiation (Jobson *et al.*, 2009).

Chk2 knockout mice and chk2-deficient cells such as non small lung cancer are remarkably resistant to IR induced apoptosis and chemotherapy drugs (Zhang *et al.*, 2004). Low level or absent of chk2 kinase promote breast, prostate, thyroid, bladder, kidney, ovarian, and colorectal cancers. Epigenetic silencing of chk2 expression is shown in lung cancer (Stolz *et al.*, 2011). In addition; Chk2 siRNA prevented the release of the antiapoptotic factor survivin from the mitochondria, thus augmenting ionizing radiation (IR)- or doxorubicin-induced apoptosis (Jobson *et al.*, 2009). The chk2-specific inhibitor,

VRX0466617 decreased IR-induced apoptosis whereas did not have the effect on the cytotoxicity of doxorubicin, taxol or cisplatin (Carlessi *et al.*, 2007) On the other hand, Chk2 knockout in colorectal cancer cells accelerate apoptosis.

Many anticancer agents induce cell death by damaging DNA including cisplatin, Adriamycin, and ionizing radiation (IR). Chk2 activation is also induced by anticancer drugs including doxorubicin (Théard *et al.*, 2001), etoposide (Mir Mohammadrezaei *et al.*, 2012), gemcitabine (Karnitz *et al.*, 2005), cisplatin (Pabla *et al.*, 2008) and ionizing radiation (Gogineni *et al.*, 2011). In the response to etoposide chk2 is activated and induce cell death in p53 dependent manner in MCF-7 cells (Zhang *et al.*, 2004) However, resistant to these drugs provides a barrier to efficacious cancer treatment (Carlessi *et al.*, 2007). Alterations of genes of involved in DNA damage pathway can modulate chemo/radio sensitivity in cancer cells.

A selective chk2 inhibitor and chk2 siRNA enhance the sensitivity of pancreatic cancer cells in combination with gemcitabine. Treatment of C3742, a chk2 inhibitor, increase efficacy of cisplatin in human ovarian cancer (Duong *et al.*, 2013). These findings show the synergistic antitumor effect by combinatorial treatment anticancer drugs with a chk2 inhibitor (Duong *et al.*, 2013). Chk2 siRNA enhance camptothecin induced apoptosis in HCT116 cells that indicate chk2 is an apoptosis inhibitor. Inhibition of chk2 reduce doxorubicin-induced G2 blockade and increase the frequency of apoptotic cells in HCT116 cells. Furthermore, chk2 provide a survival signal for tumor cells (Jobson *et al.*, 2009). The role of chk2 is various and depending on the cellular genetic background, damage forms and potency.

Thus, it is not easy to demonstrate whether chk2 inhibition should be more effective as a therapeutic approach in cancers. However, chk2 may be therapeutic targets that overcome the resistance of anticancer drugs in cancers.

Conclusion

In future more studies is necessary to identify the precise role chk2 in tumorigenesis also clinical application of chk2 inhibitor when is combined with anticancer drugs in cancer treatment. These findings may provide beneficial therapeutic approach for cancer therapy.

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