RESEARCH ARTICLE

CK2 Enzyme Affinity Against c-myc$^{424-434}$ Substrate in Human Lung Cancer Tissue

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Abstract

CK2 is a serine threonine kinase that participates in a variety of cellular processes with more than 300 defined substrates. This critical enzyme is known to be upregulated in cancers, but the role of this upregulation in carcinogenesis is not yet fully understood but c-myc, one of the defined CK2 substrates, is a well-known proto-oncogene that is normally essential in developmental process but is also involved in tumor development. We evaluated the optimal enzyme and substrate concentrations for CK2 activity in both neoplastic and non-neoplastic human lung tissues using the c-myc$^{424-434}$ peptide (EQKLISEEDL) as a substrate. The activities measured for the neoplastic tissue were 600-750 U/mg protein while those for the control tissue was in the range of 650-800 U/mg. K_m value for c-myc peptide was determined as 0.33 μM in non-neoplastic tissue and 0.18 μM in neoplastic tissue. In this study, we did not observe an increased activity in the neoplastic tissue when compared with the non-neoplastic lung tissue, but we recorded two times higher affinity for c-myc$^{424-434}$ in cancer tissue. Considering the metabolic position of c-myc$^{424-434}$, our results suggest that phosphorylation by CK2 may be important in dimerization and thus it might affect the regulation of c-myc in cancer tissues.

Keywords: Casein kinase 2 - lung tumors - c-myc - affinity

Asian Pacific J Cancer Prev, **13 (10)**, 5233-5236

Introduction

Casein Kinase 2 (CK2), a highly conserved serine/threonine kinase, participates in a number of cellular processes including cell cycle control, differentiation, proliferation, survival, apoptosis and carcinogenesis (Bibby and Litchfield, 2005; Filhol and Cochet, 2009; Dastidar, 2012). CK2 has two catalytic subunits (α, α', α'') which are linked via two regulatory (β) subunits to form a tetrameric complex (Litchfield et al., 2001; Shi et al 2001; Litchfield 2003). This complexes can be formed as different combinations of the subunits such as $\alpha_2\beta$, $\alpha\alpha'\beta_2$, $\alpha'_2\beta_2$ depending on the cell type (Trembley at al., 2010).

It is known that CK2 tends to phosphorylate serine/threonine residues specially surrounded by acidic residues. Based on this, a minimal consensus sequence for CK2 is defined as "Ser/The- X-X- Acidic" where the acidic residue might be Glu, Asp, pSer, pThr. Even though this consensus sequences have been defined as new potential substrates of the CK2, it is important to note that there are exceptions where the phosphorylation by CK2 occurs other than this consensus sequence (Meek et al., 1990; Pinna 1990; Meggio et al.,1994; Pinna et al., 1997; Litchfield, 2003).

CK2 contains more than 300 identified substrates in mammalian cells including DNA binding proteins, nuclear oncoproteins and transcription factors (Olsten et al 2004; Dastidar et al., 2012). It also combines with a number of apoptosis-related factors (Shin et al,, 2005; Kim et al., 2008), thus participates in the regulation of such molecules as procaspase 2 (Wang et al., 200), RelA/p65 (Li et al., 2002), ARC (Dasagher et al., 2001) and bid involved in cell death and survival (Trembley at al., 2009).

Up-regulation of CK2 has been reported in all cancers so far examined and it has been stated that this upregulation is due to increased protein levels. Thus, stable CK2 levels seem to be important in the homeostasis of the cell (Guerra et al., 1999; Tawfic et al., 2001; Trembley et al., 2009). It has been demonstrated that increased CK2 expression in cancer cells reflected not only proliferation but also the state of dysplasia (Trembley et al., 2009). CK2 itself is not an oncogene, its contributory oncogenic potential has been researched using experimental studies. Functional interaction of CK2 and c-myc in lymhomagenesis has been shown by Channavajhala et al.(2002) and association of CK2 in other carcinomas such as lymhoblastic leukemia (Kelliher et al., 1996) and mammary gland tumorgenesis

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(Landesman et al 2001) is also known.

Myc proto-oncogene protein (NP_002458.2) (Pruitt et al., 2009) has been reported as a substrate for CK2 in cells. CK2 phosphorylates the c-myc in the C-terminal PEST domain, in consensus sites between 240-262 and 342-429 residues, thus there may be a critical regulatory of c-myc protein stability and cell proliferation in T cell lymphomas (Lüscher et al., 1989; Penn et al.,1990; Bousset et al.,1993; Bousset et al.,1994; Channavajhala et al., 2002). c-myc is a nuclear transcription factor which is essential for the developmental processes in mammalian organisms (Channavajhala et al., 2002) and up-regulated in many cancer types. Regulation of c-myc expression is either in transcriptional and post-transcriptional levels, and any kind of disregulation may result in the transformation of cells (Penn et al., 1990; von Deimling et al., 1990; Channavajhala et al., 2002). It has been shown that myc family oncogenes are involved in carcinogenesis not only in the initiation but also in progression step by re-arrangement and amplification of myc genes. It is also stated that small cell lung tumors which has myc amplification are more aggressive and patients having this cancer have worse prognosis (Wallis et al., 1999). Increased CK2 activity in human lung tumor tissue for a specific synthetic peptide (RRRDDDSDDD) has been shown in our previous study (Yaylim and Isbir, 2002). The aim of this study is to determine the optimal enzyme and substrate concentrations in non-neoplastic lung tissue, and then compare the CK2 activity in lung cancer tissue and non-neoplastic lung tissue using c-myc peptide as the substrate to see if there is any correlation between increased c-myc levels and CK2 activity.

Materials and Methods

Tumor and non-neoplastic tissues with lung carcinoma were used for the CK activity determination. The study was approved by the Clinical Research Ethics Committee of Istanbul Faculty of Medicine, at the Istanbul University, and participating subjects were informed prior to the study. The diagnosis of lung carcinoma was confirmed pathologically. Tumor material with no or minimal inflammatory infiltrations was selected. Cell extracts (about 0.1- 0.5 g tissue) were minced and transferred to 0.5-2 ml ice-cold buffer (2 mM β-mercaptoetanol, 20 mM Tris-HCL, 0.5 mM phenyl methyl sulphonyl floride ($PhMeSO_{2F}$). The material was then homogenized with Ultrathorax (20,000 rpm) three times for 30 seconds at 30-second intervals. The homogenate was sonicated four times for 15 seconds at 10-second intervals at 50 W using a Branson sonifier with a microtip. The sonicated material was then centrifuged at 39,000 g and 4°C for 2 hours in a Sorval ultracentrifuge. The protein content was determined by the Lowry method (Dobrowolska et al., 1999) and concentrations adjusted to 1 mg/ml with buffer B (20 mM Tris-HCL, 100 mM NaCl, 0.5 mM $PhMeSO_4F$, 7 mM 2-mercaptoetanol pH: 7.2). This extract was used for the CK2 activity test.

Determination of CK2 activity and kinetic studies

Casein Kinase 2 activity was measured as described previously (Krippner-Heidenreich et al., 2001). The c-myc$^{424-434}$ peptide (EQKLISEEDL) is used in concentrations of 0.5 μM, 1 μM, 1.5 μM, 10 μM. In our assay, the mixture of the phosphatase inhibitors ammonium vanadate (0.2 mM) and NaF (50 mM) appeared to influence the magnitude of the CK2 activity in the extracts. One unit of CK2 activity is defined as the amount that catalyses the transfer of 1 pmol ^{32}P into the substrate within 1 minute at 37°C. The Lineweaver Burk and Hill Plot were constructed.

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  1  mdffrvvenq qppatmplnv sftnrnydld ydsvqpyfyc deeenfyqqq qqselqppap
 61  sediwkkfel lptpplspsr rsglcspsyv avtpfslrgd ndggggsfst adqlemvtel
121  lggdmvnqsf icdpddetfi kniiiqdcmw sgfsaaaklv seklasyqaa rkdsgspnpa
181  rghsvcstss lylqdlsaaa secidpsvvf pyplndsssp kscasqdssa fspssdslls
241  stesspqgsp eplvlheetp pttssdseee qedeeeidvv svekrqapgk rsesgspsag
301  ghskpphspl vlkrchvsth qhnyaappst rkdypaakrv kldsvrvlrq isnnrkctsp
361  rssdteenvk rrthnvlerq rrnelkrsff alrdqipele nnekapkvvi lkkatayils
421  vqAEEEQKLIS EEDLLRKRRE QLKHKLEQLR NSCA
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Figure 1. Represents the c-myc$^{424-434}$ Peptide within the "Helix-loop-helix" and "leucine zipper" Domains of the Myc. Underlined residues indicate the dimerization interface of Myc. Grey area, residues between 370 and 426, indicates the "Helix-loop-helix" domain. Residues high-lighted in capital letters, between 423 and 454 indicate "leucine zipper"domain. Underlined residues indicate dimerization interface. Residues high-lighted in bold capital letters indicate c-myc$^{424-434}$ peptide

Table 1. Represents the Kinetic Values of the Ck2 Enzyme When c-myc$^{424-434}$ Peptide is used as Substrate

	Vmax (pmol/min/mg)	K_m (uM)
Lung cancer tissue	666	0.18
Non-neoplastic	714	0.33

Results

The Michealis Menten kinetic values for CK2 are calculated for c-myc$^{424-434}$ peptide (EQKLISEEDL) in both lung carcinomas and non-neoplastic lung tissue of the same patient. The activities measured for the neoplastic tissue were in range of 600-750 U/mg protein while those for the control tissue were in the range of 650-800 U/mg. K_m value for c-myc peptide was determined as 0.33 μM in non-neoplastic tissue and 0.18 μM in neoplastic tissue.

Discussion

CK2 is a pleiotropic protein kinase which phosphorylates serine/threonine residues and it can be stated that CK2 regulation is highly related with carcinogenesis, since it's up-regulation has been reported in all cancers that have been examined so far (Trembley et al., 2009; Dastidar et al., 2012). Nuclear transcription factor c-myc is one of the defined substrates of CK2 which is known to be upregulated in most of malignancies (Münstermann et al.,1990; Daya-Makin et al., 1994; Gapany et al., 1995; Landesman-Bollag et al., 2001; Channavajhala et al 2001;). Even though c-myc had been defined to phosphorylate by CK2 in PEST domain, neither the consequences of this phosphorylation nor other possible CK2 phosphorylation sites in c-myc has become clear yet (Channavajhala et al., 2001; Bousset et

al.,1993; Bousset et al., 1994; Lüscher et al.,1989; Street et al., 1990).

According to "Human Protein Reference Database" there are 72 possible CK2 motifs in the c-myc protein (Amanchy et al., 2007). Among these 72 motives, only a restricted number of c-myc peptides have been used as the substrates of the CK2 in enzyme activity studies. Bernard et al. (1989) defined the CK2 phosphorylation sites of the avian myc peptides as myc$^{222-238}$ (K_m:10.43 μM), myc$^{246-263}$ (K_m: 20 μM), myc$^{323-334}$ (K_m: 3920 μM). They also showed the human myc peptides homologus for avian myc$^{222-238}$ and myc$^{323-334}$: myc$^{240-262}$ (LHEETPPTTSSDSEEEQEDEEEI) and myc$^{342-357}$ (CTSPRSSDTEENVKRR) respectively and stated that the phosphorylation sites of the human myc by CK2 were located within residues 240-262 and 342-357 (Lüscher et al., 1989). Dobrowolska et al. (1999) used the c-myc peptide as a substrate for CK2. They examined the differences in the kinetic properties of CK2 holoenzymes $\alpha 2\beta 2$ and $\alpha' 2\beta 2$ with synthetic peptide (RRRDDDSDDD) and c-myc peptide (RRRPPTTSSDSEEEQEEDEE).

In our study, we used the c-myc peptide (EQKLISEEDL) myc$^{424-434}$ as a substrate for CK2 in lung cancer tissue and non-neoplastic lung tissue. The aim of the study is to determine if there is any correlation between increased c-myc levels and CK2 activity in lung cancers. As it is shown in Figure 1, the myc$^{424-434}$ peptide is in the range of leucine zipper domain (423-454) which is efficient for DNA binding. It is also a part of Helix-loop-Helix domain (370-426) which is a specific sequence for DNA binding proteins that act as a transcription factor and polypeptide binding site "dimerization interface" (424-425) (Pruitt et al., 2009).

Since we knew the existence of a phosphorylation motive for CK2 and of its critical position, we decided to use c-myc$^{424-434}$ peptide as a substrate. We did not observe a significant difference in the CK2 activity between neoplastic and non-neoplastic tissues. Vmax value in cancer tissue was 666 pmol/min/mg protein while Vmax in non-neoplastic tissue was 714 pmol/min/mg protein (Table 1). However, we observed an interesting change in K_m values. In the non-neoplastic tissue, K_m value was higher than the lung cancer tissue; 0.33 μM and 0.18 μM respectively. This change in the K_m value indicates that affinity of the CK2 for c-myc peptide in cancer tissue is two times higher. In order to explain this increase in the affinity of the enzyme, even though the activity remained the same, we focused on the critical position of the c-myc$^{424-434}$. As it is stated above, EQKLISEEDL peptide sequence is just between the functionally important and conserved domains HLH and Leucin Zipper domains of the c-myc. Moreover, c- myc$^{424-434}$ is really close to one of the defined "polypeptide binding sites" where c-myc binds to its "binding partner" MAX (Figure 1) (Pruitt et al., 2009).

CK2 is known to phosphorylate MAX in vitro and affect the DNA binding properties of both MAX/MAX and MYC/MAX dimers (Litchfield, 2003). Moreover, Max is known to be protected from caspase mediated cleavage when phosphorylated by CK2 (Krippner-Heidenreich et al., 2001; Litchfield, 2003). It has been shown that DNA binding capability of MAX/MAX dimers are reduced by CK2 phosphorylation of MAX, whereas MAX/MYC dimers remained unaffected (Berberich et al.,1992).

If we take the data together, CK2 both phosphorylates MYC and its binding partner MAX and affects the DNA binding capacity of MAX/MAX dimers (Litchfield, 2003). Moreover, phosphorylation of MAX by CK2 protects it from caspase cleavage. Based on this information, it is possible to conclude that CK2 phosphorylation of myc might have a regulatory role, specifically on the dimerization and/or DNA binding.

Our findings indicate that c-myc$^{424-434}$ peptide is phosphorylated by CK2 in both cancer and non-neoplastic lung tissue. If we consider the position of the c-myc$^{424-434}$' peptide, this phosphorylation might be important for c-myc activity. Phosphorylation site that we've defined here is really close to the polypeptide binding site that myc interacts with the MAX. Since it has been stated that MYC-MAX binding occurs via charged /polar interactions (Brownlie et al., 1997; Amati et al., 2001; Ponzielli et al., 2005), phosphorylation of such a close residue to the binding point might have a serious impact on the formation of dimers.

In our previous study, we showed that CK2 activity is 2.5 times higher in lung cancer tissue when RRRDDDSDDD peptide is used as substrate (Yaylim and Isbir, 2002). In this study, though we did not record an increase in CK2 activity in cancer tissue, there was an obvious increase in the affinity of the CK2 to the c-myc$^{424-434}$ peptide. Based on the position of the peptide, we hypothesize that phosphorylation of c-myc$^{424-434}$ might affect the MYC-MAX dimerization, which is a possible explanation for the increased CK2 affinity. We believe that CK2 has an undeniable effect on c-myc activity whether it is direct or indirect. In a previous study, which was carried out by Channavajhala et al. (2002), it was stated that regulation of c-myc levels in the lymphoma cell lines could be related to the CK2 activity (Channavajhala et al., 2002). These results also support our hypothesis about the effect of the CK2 in c-myc regulation, yet the mechanism of this regulation still remains a puzzle.

In this study, we have shown that CK2 has an increased affinity for c-myc$^{424-434}$ peptide in lung cancer tissue. We assume that phosphorylation on such a critical position can influence the dimerization of the myc , thus affecting its oncogenic activity. However, further investigation is needed to confirm this hypothesis.

Acknowledgements

This study was supported by grant from the University of Istanbul, Research Foundation (project T-554/240698). Turkey.

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