

## Targeting the subunits of Protein -kinase CK2 in cancer therapy

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Protein-kinase CK2 is a highly ubiquitous and multifaceted serine/threonine kinase described as a multisubunit holoenzyme generated by the tight association of two  $\alpha$  or  $\alpha'$  catalytic subunits with a dimer of  $\beta$  regulatory subunits (1). However, the transient nature of this complex has been recently highlighted by the elucidation of its structure and the analysis of the spatiotemporal organization of its individual subunits in living cells (2, 3).

Aberrant activation of protein kinases is a key oncogenic force underlying human tumorigenesis. Traditionally, CK2 has been regarded as a constitutively active protein-kinase in search of specific cellular functions (4). However, several studies have indicated that CK2 is a stress-activated kinase that plays a crucial role in the regulation of cell proliferation and in the transduction of survival signals (5, 6). Recently, several molecular pathways modulating the prosurvival properties of CK2 have started to emerge:

- CK2 is activated by UV radiation in a p38 MAPK-dependent manner, leading to the phosphorylation and degradation of the NF $\kappa$ B inhibitor I $\kappa$ Ba (7). Upon UV irradiation, CK2 also complexes and phosphorylates p53 at Ser389. Conversely, WT p53 inhibits CK2 activity (8), supporting the notion that p53 and CK2 are interconnected in a tightly regulated network.
- CK2 is frequently activated in human cancers and can induce mammary tumors and lymphomas when expressed in transgenic mice (9, 10).
- Altered CK2 activity in human cancers leads to phosphorylation and degradation of the tumor-suppressor protein PML through integration of upstream p53 and p38MAPK signals (11).
- Hypoxia-induced activation of HIF-1 in cancer cells is mediated by a CK2-dependent downregulation of p53 (12)
- A moderate down regulation of nuclear-associated CK2 $\alpha$  leads to growth arrest and induction of apoptosis in prostate cancer cells (13) and CK2 $\alpha$  nuclear localization is associated with poor prognostic factors in human prostate cancer (Laramas et al. submitted).

Thus, there are good reasons to believe that CK2 has causal relevance to cancer and this kinase has entered into consideration as a suitable target for cancer therapy.

Importantly, it has been reported that antisense RNA-mediated CK2 $\alpha$  down regulation induces potent apoptosis in cancer cells, but minimal cell death in normal cells (13). These observations indicate that CK2 should have disease-associated functions which are separate from its normal functions. This raises the possibility of a pharmacological window in targeting CK2 for induction of apoptosis in cancer cells under conditions that may spare normal cells.

Given the subcellular dynamic of CK2 subunits and the transient nature of their interactions in living cells, the use of potent and specific inhibitors is the first-choice approach to manipulate this kinase. However, the development of such molecules has remained limited in the absence of relevant *in vivo* models useful for screening for molecules capable of modulating CK2 activity. In addition, the few molecules described, capable of inhibiting CK2 activity are ATP analogs, such as TBB (14), IQA (15) and condensed polyphenolic derivatives (16) have the drawback of being either not very specific or not very active.



for increasing binding to CK2. Collectively, these data allow the definition of a pharmacophore in Quinobene.

To check if Quinobene which efficiently inhibits CK2 *in vitro* is also efficient on it *in vivo*, non-small cell lung cancer cells (A549) were treated with increasing concentrations of TBB or Quinobene for 48h and the cell viability was monitored. The data indicate that Quinobene is cell permeable and its potency correlates with proapoptotic efficacy.

## 2) Disruptors of CK2 assembly.

CK2 catalytic subunits possess a constitutive activity. However, in eukaryotic cells, the CK2 $\beta$  subunits are central components of the tetrameric CK2 complex are more than assembly factors holding the two CK2 $\alpha$  subunits in proximity. They are also responsible for the recruitment of CK2 substrates, thereby operating as a targeting subunits and/or a docking platform affecting the accessibility to the catalytic site of binding substrates whose phosphorylation is either stimulated or prevented by the CK2 $\beta$  (20). Thus, the dynamic interaction of the CK2 subunits observed in living cells, may have a key role in CK2 signaling pathways and drugs that specifically target this interaction are less likely to have side effects than drugs that act as general inhibitors of CK2 catalytic activity. When added together *in vitro*, recombinant  $\alpha$ - and  $\beta$ -subunits assembled instantaneously into a stable heterotetrameric complex with high affinity (dissociation constant (Kd) = 5.4 nM). However, the intersubunit flexibility suggested by X-ray crystallography studies and live-cell fluorescent imaging raises the possibility that CK2 tetramers may be subjected to disassembly and re-assembly. The crystal structure of the CK2 holoenzyme has revealed the predominant contribution of the  $\alpha/\beta$ -tail contact which connects the CK2 $\alpha$  subunit with the tail of a CK2 $\beta$  monomer, for the stability of the CK2 holoenzyme. This prompted us to focus our attention on the CK2 $\alpha$  CK2 $\beta$  interface. Using alanine scanning mutagenesis, we show that only a small set of primary hydrophobic residues of CK2 $\beta$  which contacts at the center of the interface dominate affinity. A conformationally constrained peptide derived from the CK2 $\beta$  carboxyterminal domain was designed and tested in an *in vitro* CK2 subunit binding assay. This eight residues peptide (Pc) can efficiently block the formation of the CK2 holoenzyme (IC<sub>50</sub> = 3  $\mu$ M) but also led to an almost complete disassembly of the preformed complex (IC<sub>50</sub> = 6.5  $\mu$ M). Noteworthy, a linear form of the peptide was completely inactive in this binding assay showing that the constrained conformation of Pc is essential for its antagonist activity.

We next generated a set of mutant CK2 $\beta$  to test them by quantitative binding analysis. The data show that the CK2 $\alpha$  binding activity of the single mutant F190A (CK2 $\beta$ -F) was dramatically reduced and this activity was completely abrogated for the Y188A/ F190A double mutant (CK2 $\beta$ -YF) and for the M166A/ Y188A/ F190A triple mutant (CK2 $\beta$ -MYF). Thus, deciphering these protein-protein interaction sites has revealed hot spots for the molecular recognition between the two subunits and provides a deep insight into the structural determinants required to antagonize the interaction between the CK2 subunits. This knowledge was then exploited for a virtual high-throughput screening approach to isolate small chemical inhibitors of this protein-protein interaction. Several molecules belonging to the same cluster were identified and shown to inhibit at least *in vitro* the assembly of the CK2 complex. These compounds represent the first small molecules that bind to the CK2 subunit interface and inhibit the high affinity interaction of its subunits. Thus, it seems that time is ripe for CK2 to join its protein-kinase relatives as a potential therapeutic target. The identification of new inhibitors targeting differentially the CK2 subunits will guide the generation of potent and tumor-specific drugs.

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