CDKN2 modulation of CDK4 alters target engagement of CDK4 inhibitor drugs

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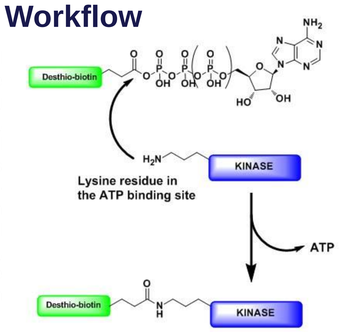
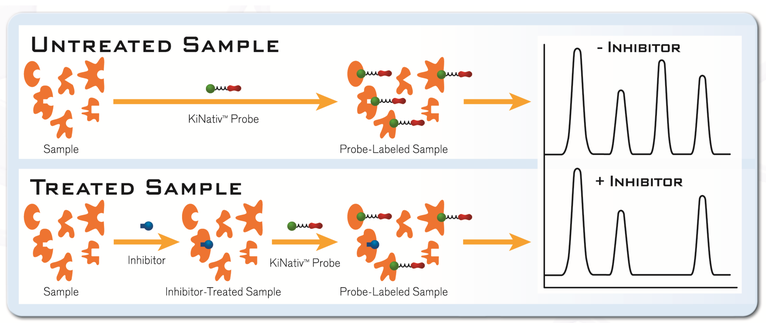
Abstract

A critical factor in achieving drug efficacy is target engagement in relevant cells, which is influenced by parameters such as pharmacokinetics and permeability. We recently provided an example of cell-type specific target engagement where the CDK4 inhibitor palbociclib engaged its kinase target in sensitive cells, but not in resistant cells[1]. Here, we describe the mechanism for the observed cell-type specific target engagement: it is determined by the interaction of the kinase with a cellular proteinaceous inhibitor of that kinase. Since both the drug and the inhibitor protein block kinase activity, discrimination between these modes of inhibition is not possible by evaluating biochemical kinase activity and was instead evaluated using a chemo-proteomics approach. We show that CDK4 target occupancy is high in cells that lack the CDK4 inhibitor protein CDKN2A or harbor a mutation in CDKN2A that prevents binding to CDK4. Conversely, in cells with wildtype CDKN2A or transgenic expression of any CDKN2 family protein, the CDK4 nucleotide binding site is altered such that CDK4 no longer reacts with a nucleotide probe. CDKN2A knockdown rescues binding of CDK4 to probe and to palbociclib. In cells with a CDK4-R24C mutation that reduces CDK4 interaction with CDKN2A, active CDK4 retains labeling with nucleotide probe and displays reduced interaction with palbociclib. This work illustrates the impact of cellular context on target engagement with kinase inhibitors.

KiNativ® Workflow

The addition of the ATP or ADP acyl phosphate desthiobiotin probe (nucleotide probe) to cellular lysates results in probe binding to the active site of protein and lipid kinases, followed by a covalent reaction with conserved lysine residues in the active site[1,2]. In cases where a compound is bound to a kinase, probe-labeling is inhibited. The sample is then denatured, reduced, alkylated, gel-filtered and trypsinized. Probe-labeled peptides are captured, eluted, and analyzed by mass spectrometry. The basic workflows for the experiments described in this study are shown below.





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7. **Raspé, E et al., CDK4 phosphorylation status and a linked gene expression profile predict sensitivity to palbociclib. EMBO Molecular Medicine, 2017.**
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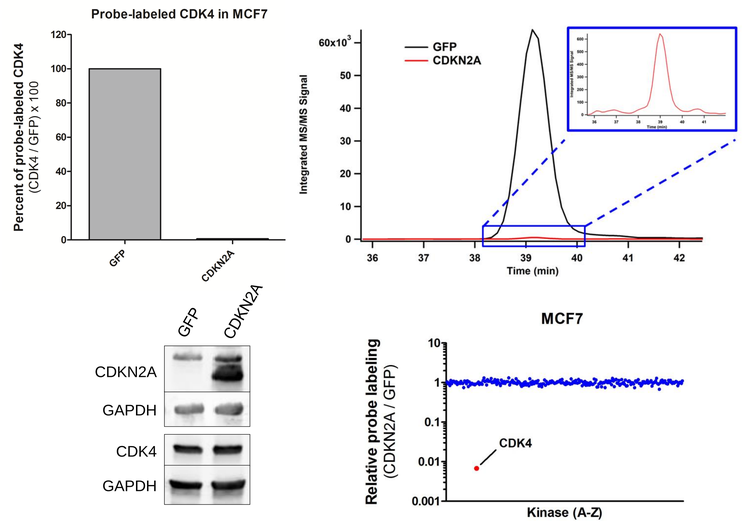
CDKN2A expression reduces CDK4-probe labeling



Transgenic expression of CDKN2A in the CDKN2A-null cell line MCF7 reduced the MS signal of CDK4 >90%; no other kinase signals were inhibited. Expression of the other CDKN2 family proteins, but not the CDKN1 family proteins, also inhibited CDK4 probe-labeling (not shown).

Residual probe-labeled CDK4 is not inhibited by CDK4 inhibitor drugs

	MCF7 lysate					
	0.5 µM Palbociclib		0.5 µM Ribociclib		0.5 µM Abemaciclib	
Symbol(s)	Txn GFP	Txn CDKN2A	Txn GFP	Txn CDKN2A	Txn GFP	Txn CDKN2A
CDK4	87	11	68	1	93	30
CDK6	79	-14	62	-10	83	-9
CaMK2b	1	11	41	57	91	91
CaMK2d	9	29	53	73	93	95
CaMK2g	7	14	64	63	96	96
CaMKK2	12	15	15	23	76	79
CDK9	11	11	12	4	58	53
GSK3A	-3	-7	-3	-1	88	86
GSK3B	-2	-7	4	-2	74	74
ICK	-5	-6	-12	-15	51	54
IRAK1	-12	9	-3	24	89	91
JNK1, JNK3	29	15	16	-2	66	60
JNK2	40	38	18	-1	55	55
PCTAIRE1	48	49	15	28	84	81
PCTAIRE2	58	54	27	21	89	88
PFTAIRE1	11	16	3	0	47	55
PIK3C3	53	63	7	25	51	67
PIP4K2C	57	68	17	39	56	65
PIP5K3	6	22	-17	26	66	73

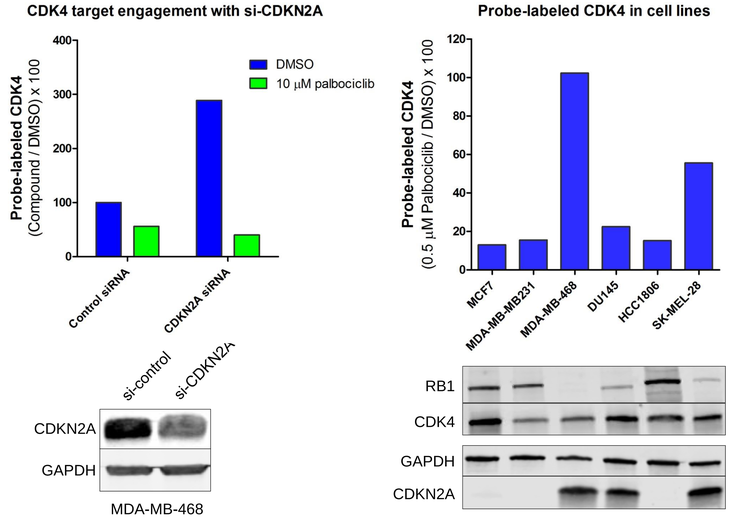
Legend

- >90% Inhibition
- 75 - 90% Inhibition
- 40 - 75% Inhibition
- <40% Inhibition

Lysates from CDKN2A-expressing MCF7 cells were treated with CDK4 inhibitor drugs. At 0.5 µM, neither palbociclib, ribociclib nor abemaciclib inhibited CDK4 probe-labeling while off-target inhibition profiles were not affected.

CDKN2A-CDK4 interaction determines palbociclib target engagement

Palbociclib-CDK4 target engagement is minimally observed In MDA-MB-468 cells, which express wildtype CDKN2A and are resistant to growth inhibition by palbociclib[3]. siRNA knockdown of CDK2NA resulted in a three-fold increase in probe-bound CDK4. Palbociclib inhibited probe-binding of this newly exposed fraction of CDK4, suggesting that release from CDKN2A enables CDK4 reactivity with both probe and small molecule inhibitors.



Palbociclib-CDK4 target engagement was assayed in cell lines with genetic backgrounds representing naturally-occurring alterations to the CDK4-CDKN2A interaction. Probe binding was inhibited in the MCF7 and MDA-MB-231 cell lines that harbor genomic deletion of CDKN2A[4] and in DU145 cells that carry a D84Y mutation in CDKN2A that disrupts binding to CDK4[5]. In contrast, no target engagement was observed in MDA-MB-468 cells that express wildtype CDKN2A. The SK-MEL-28 cell line has an R24C mutation in CDK4 that reduces binding to CDKN2A but does not reduce kinase activity[6]. Target engagement was intermediate in these cells, presumably due to residual CDK4-CDKN2A interaction that reduced the affinity of CDK4 for palbociclib. HCC1806 cells are reported to be resistant to palbociclib due to Cyclin E-mediated bypass of CDK4[7,8]. Consistent with this mechanism of resistance, CDK4 target engagement was observed in HCC1806.

Conclusions

While not always sufficient, target engagement is necessary for drug response. As shown here, kinase target engagement becomes dependent on the cellular context when nucleotide binding affinity, and by extension affinity for ATP-competitive drugs, is modulated by endogenous inhibitors of the kinase. Therefore, evaluation of the integrity of the nucleotide binding pocket of drug targets in multiple biologically relevant samples should facilitate predictions of drug response, especially in cases where the compendium of endogenous cellular inhibitors is not fully understood.

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