Selective mutation in ATP-binding site reduces affinity of drug to the kinase: a possible mechanism of chemo-resistance

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"Letter to the editor"

Selective mutation in ATP binding site reduces affinity of drug to the kinase: a possible mechanism of chemo-resistance

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Mutation in protein kinases is very common in cancer and causes constitutive activation of protein kinases resulting in hyper activation of survival pathways. Recent studies suggest that mutation in ATP binding site of kinases confers resistance to the chemotherapy [1]. Thus, understanding how mutations reverse to the drug will be beneficial for effective drug development against cancer. We explored this issue using a family of protein serine/threonine kinases as a model. The protein kinase C (PKC) family of protein serine/threonine kinases consists of 10 proteins encoded by 9 genes which is known to involve in many cancers [2]. We first modeled kinase domain structure of all 10 PKC isoforms using SWISS-MODEL and further verified using ProSA. Modeled structures were processed for energy minimization in a water cube using GROMACS. Autodock4 was used for docking inhibitors in kinase domains. Initially we used PDK1 kinase domain with LY333531 to validate our system. We observed that our modeled structure docked with LY333531 perfectly overlapped with X-Ray structure of PDK1 kinase domain and LY333531 complex (Fig. 1A) suggesting that our method is reliable. Furthermore, we docked LY333531 in PKCβ2 kinase domain and observed perfect docking of inhibitor in ATP binding site (Fig. 1B-D). Three residues (K, D, D) of VAIK, HRD and DFG motifs are catalytically important and highly conserved in eukaryotic protein kinases. Threonine in activation loop is also highly conserved in PKC and also some AGC kinases. Though PKC family is divided into three sub-families [2], we asked the question that whether conserved residues are also structurally conserved within the family. To address this question, we determined torsion angels of respective residues and observed that catalytically important residues are

also structurally well conserved (Fig. 1E). Though we found that catalytically important residues are structurally well conserved across the family, we set out to determine inhibitor binding residues across the 10 kinase domains of PKC isoforms. LY333531 was used as a ligand to determine inhibitor interacting residues. Docking studies identified 14 residues which are critical for the interactions (Table S1) and these residues are also well conserved within the family (Fig. 1F). Then, we determined whether inhibitor interacting residues are common for other kinase inhibitors. PKCβ2 was used as a macromolecule to dock nine different kinase inhibitors. We observed that most of residues are common for the interaction (Table S2) suggesting similar mechanism is involved in inhibition. Thus, we suggest that conventional kinase inhibitors are designed to interact within the similar binding pocket. Since all 10 isoforms share similar residues for interaction with inhibitors we used PKCβ2 kinase domain to determine importance of individual residues involved in interaction with inhibitors. We replaced eight individual residues with glycine and determined binding energy with LY333531 using Autodock4. We observed that mutation in any of those residues decreases binding energy and increases inhibitory concentration (Table S3). Taken together our results suggest that conventional kinase inhibitors targets similar binding pocket in ATP binding site of kinase domain and mutation in this pocket results in resistance to the inhibitor which is often observed in cancer patients [3].

Conflict of interest:

The authors declare no conflict of interest.

References

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Figure legend:

Fig. 1: Docking of inhibitor in kinase domain: (A) Comparison of modeled structure with X-Ray structure. (B) Docking LY333531 in PKCβ2 kinase domain. (C) Mesh structure of LY333531 in PKCβ2 kinase domain. (D) Structure of LY333531 in PKCβ2 kinase domain showing interacting residues. (E) Torsion angles of critical residues conserved in kinase domains. (F) Torsion angles of residues involved in interaction with ligand.

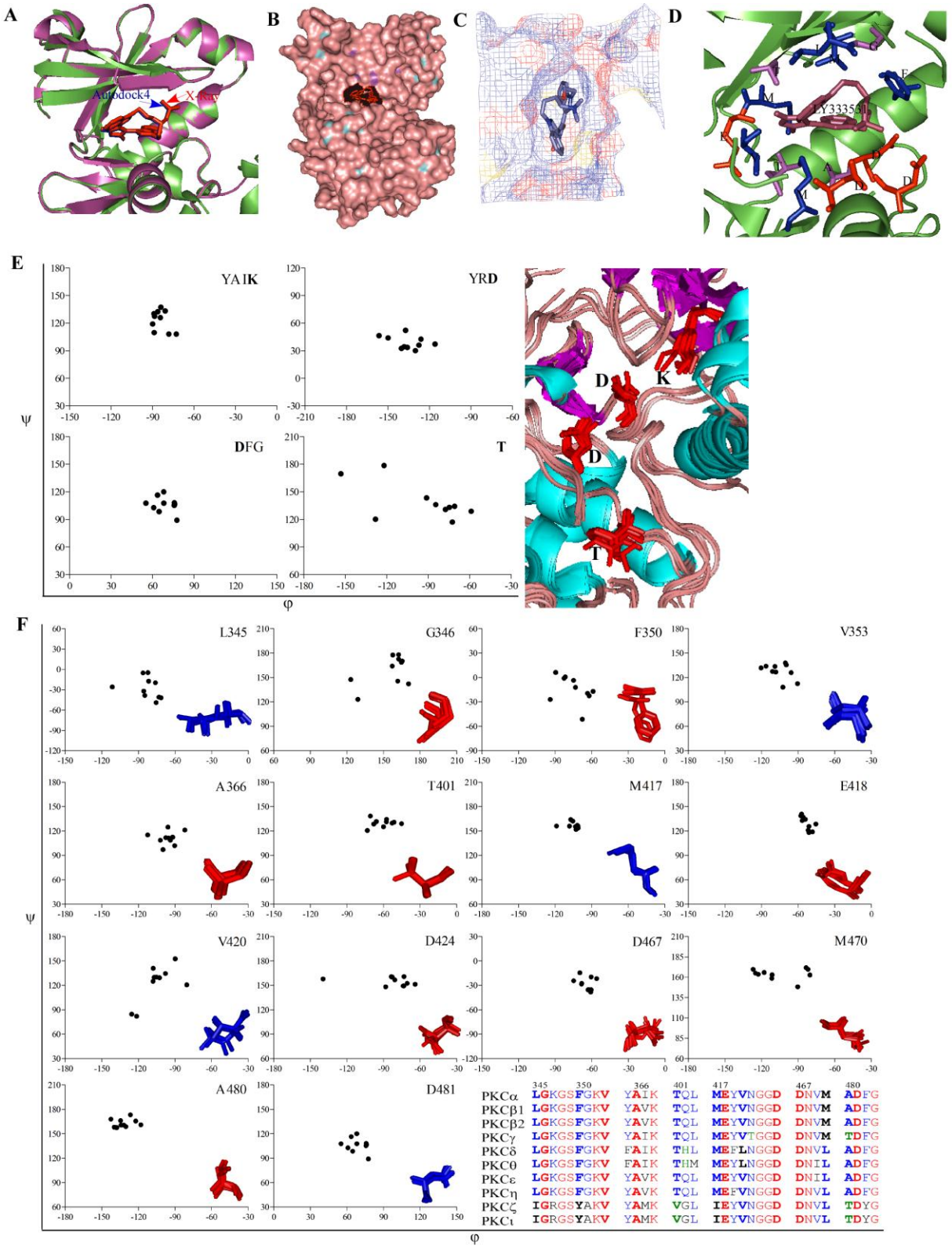
A
Autodock4
X-Ray
B
C
D
E
YAIK
YRD
DFG
T
ψ
φ
D
K
D
I
F
L345
G346
F350
V353
A366
T401
M417
E418
V420
D424
D467
M470
A480
D481
PKCα LGKGSFGKV YAIK TQL MEYVNGGD DNVM ADFG
PKCβ1 LGKGSFGKV YAVK TQL MEYVNGGD DNVM ADFG
PKCβ2 LGKGSFGKV YAVK TQL MEYVNGGD DNVM ADFG
PKCγ LGKGSFGKV YAIK TQL MEYVTGGD DNVM TDFG
PKCδ LGKGSFGKV FAIK THL MEFLNGGD DNVL ADFG
PKCθ LGKGSFGKV FAIK THM MEYLNGGD DNIL ADFG
PKCε LGKGSFGKV YAVK TQL MEYVNGGD DNIL ADFG
PKCη LGKGSFGKV YAVK TQL MEFVNGGD DNVL ADFG
PKCζ IGRGSYAKV YAMK VGL IEYVNGGD DNVL TDYG
PKCι IGRGSYAKV YAMK VGL IEYVNGGD DNVL TDYG

Figure 1

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Supplementary tables

Table S1: Residues required for interaction with inhibitor.

PKC	Leu	Gly	Phe	Val	Ala	Thr	Met	Glu	Val	Asp	Asp	Met	Ala	Asp
α	L345	G	F350	V	A366	T	M417	E418	V420	D	D467	M470	A480	D481
β1	L348	G	F353	V356	A369	T404	M420	E421	V423	D	D470	M473	A483	D484
β 2	L348	G349	F353	V356	A369	T404	M420	E421	V423	D	D	M473	A483	D484
γ	L357	G358	F362	V	A378	T418	M434	E435	V437	D	D	M487	T497	D
δ	L355	G356	F360	V363	A376	T411	M427	E428	L430	D434	D477	L480	A490	D491
ε	L414	G	F419	V	A	T470	M	E487	V489	D493	D536	L539	A549	D550
η	L361	G	F366	V369	A382	T417	M433	E434	V436	D	D483	L486	A496	D497
θ	L386	G387	F391	V394	A	T	M458	E	L	D465	D508	L511	A	D522
ζ	I	G259	Y	V266	A279	V	I330	E331	V333	D337	D380	L383	T393	D
ι	I260	G261	Y	V268	A281	V	I332	E	V335	D	D382	L385	T395	D396
PDK1	L88	G	F	V96	A109	V143	L159	S160	A162	E	E	L212	T222	D223

Table S2: Residues required for interaction with different inhibitors.

Inhibitor	Leu 348	Gly 349	Phe 353	Val 356	Ala 369	Glu 390	Thr 404	Met 420	Glu 421	Tyr 422	Val 423	Asp 427	Asp 470	Met 473	Ala 483	Asp 484
AEE788			S	S	S	S		S	B		S		S	S		B S
BisindolylmaleimideI	B S	B		S	S		S		B		B	B S	B	S	S	
LY333513	B S	B	S	S	S		S	S	B		B			S	S	B S
Dasatinib	S		S	S	S		S	S		S	B	S	S	S		
Enzastaurin	B S	B	S	S	S					S	B S	S	S	S	S	
Lapatinib			S	S	S	S		S	B	B S	B S			S		B S
Nilotinib	B	B	B S	S	S	S	S	S	B					S		B S
Pegaptanib	B S	B	S	S		S	S				B S	S		S	B S	B

Staurosporine	S		S		S		S	S	B	B S	B S		B	S	S	B S

Table S3: Difference in binding energy for mutants.

Mutant	Binding energy	KI (nM)	Distance from WT (A)
WT	-10.56	18.18	
F353G	-10.03	44.66	0.059
V356G	-9.54	101.59	0.005
A369G	-10.32	27.42	0.039
T404G	-10.11	39.09	0.010
M420G	-9.98	48.23	0.047
M473G	-9.91	54.84	0.046
D484G	-9.8	66.03	0.025