Dipeptidyl Peptidase III Cancer Mutations

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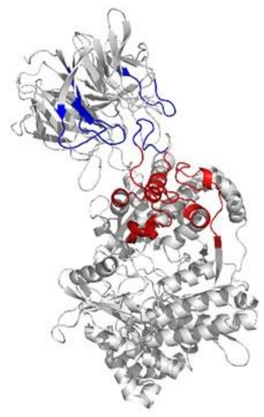
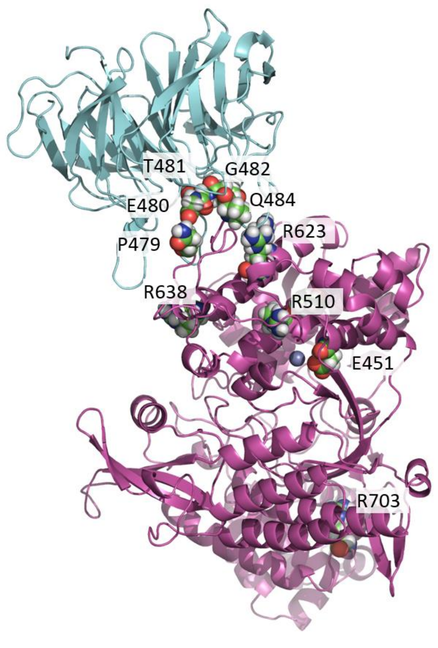


Introduction

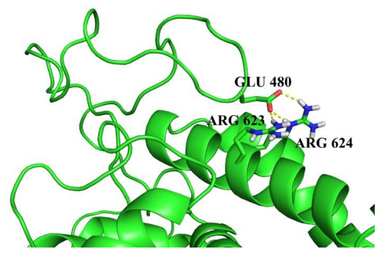
- Kelch-like ECH-associated protein 1 (KEAP1) is a cellular sensor of oxidative stress and a negative regulator of nuclear erythroid 2-related factor 2 (NRF2) [1].
- Human dipeptidyl peptidase III (DPP III), a cytosolic metallopeptidase, interacts with the Kelch domain of KEAP1 [1] via the ETGE motif located in a flexible loop of the upper domain. We investigated the impact of mutations listed in the cBioPortal for Cancer Genomics on the affinity of DPP III for the Kelch domain and on the KEAP1-NRF2 pathway.

Results

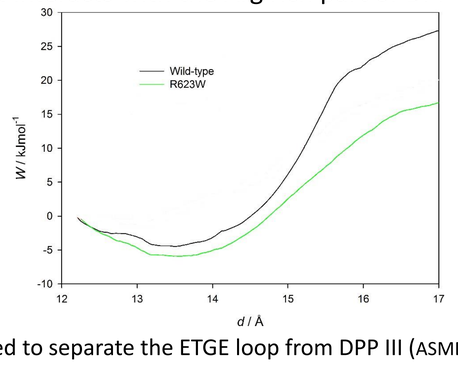
DPP III mutations



DPP III loop - locked



The ETGE motif is attached to the DPP III 'body' by strong H-bonds, through which it interacts with the Arg rich part of DPP III [2].



Work required to separate the ETGE loop from DPP III (ASMD simulations).

MST measurements

Binding affinity of DPP III mutants for the Kelch domain compared to the affinity of the wild-type protein, expressed as the ratio K_d(WT)/K_d(mutant). Measurements were performed in triplicate [3].

DPP III	K_d(WT)/K_d(mut) [a]
WT	1.0
E451K	2.1
P479S	**18.4**
E480Q	0.1
T481M	0.1
G482C	0.8
Q484H	2.1
R510W	0.3
R623W	**160.0[b]**
R638L	2.0
R638W	2.0
R703C	1.7

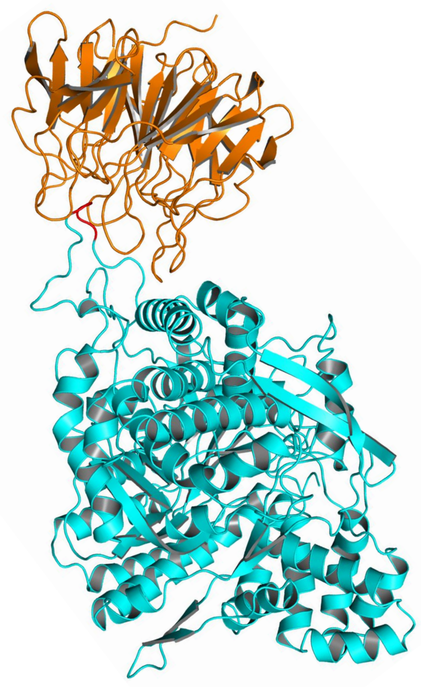
[a] The average error for the MST measurements is about 30%
[b] Matić et al., 2021 [2]

MM/GBSA energies calculated during 700 ns of MD simulations

DPP III	MMGBSA (kcal/mol)^{min}
WT	-80
P479S	-116
E480Q	-42
T481M	-63
G482C	-56
R510W	-69
R623W	-65*

*The interaction energy between DPP III and the Kelch domain decreased in this mutant, but due to the easier release of the flexible loop from the protein body, the binding affinity increased.

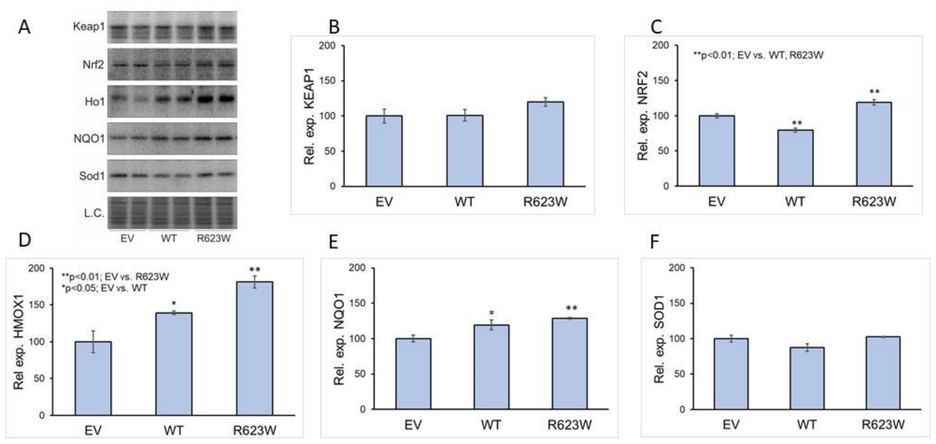
The lowest-energy structure of the DPP III (cyan) – Kelch (orange) complex with the ETGE motif in red.



- Binding of DPP III to KEAP1 is a two-step process involving endergonic translocation of the loop, followed by exergonic interactions between DPP III and the Kelch domain.

Western blot analysis

Binding of DPP III to KEAP1 results in the release of NRF2 from the complex with KEAP1 and activation of genes involved in the oxidative stress response controlled by NRF2.



- Western blot analysis of the relative expression of KEAP1, NRF2, HMOX1 (Ho1), NQO1 and SOD1 in the cells transfected with EV, WT and R623W, respectively. (A), A graphical display of the averaged densitometry values obtained by the analysis of the western blot bends in ImageLab v. 6.1.0. normalized to loading control (L.C.) KEAP1 (B), NRF2 (C), HMOX1 (E), NQO1 (D) and SOD1 (F) protein expression. (NRF2: ** $p < 0.01$ EV vs. WT, ** $p < 0.01$ EV vs. R623W; HO1: * $p < 0.01$ EV vs. R623W, ** $p < 0.05$ EV vs. WT; NQO1: * $p < 0.01$ EV vs. R623W, ** $p < 0.05$ EV vs. WT).

Conclusions

- **A multidisciplinary approach revealed that DPP III binds to the Kelch domain of the KEAP1 protein with similar affinity to NRF2, suggesting possible involvement of DPP III in the NRF2-KEAP1 pathway. The ETGE motif was confirmed to be critical for the interaction between DPP III and the Kelch domain.**
- **Mutations of DPP III listed in the cBioPortal for Cancer Genomic Database affect affinity of DPP III for the Kelch domain of KEAP1 protein, and KEAP1-NRF2 signalling pathway. The R623W and P479S mutations increase the affinity of DPP III for KEAP1 by affecting the different phases of binding of DPP III to KEAP1: Mutation R623W facilitates the detachment of the ETGE loop from the structured part of the upper DPP III domain, and mutation P479S increases the strength of the interaction between the DPP III loop and the Kelch domain of KEAP1 [3].**
- **In addition, R623W was shown to increase the expression of some NRF2-controlled genes such as HMOX1 [3]. Our results suggest that it is important to study the mutations of DPP III found in cancer to gain more insight into the mechanism of their possible involvement in cancer progression.**

Acknowledgements

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References

[1] Hast, B.E.; Goldfarb, D.; Mulvaney, K.M.; Hast, M.A.; Siesser, P.F.; Yan, F.; Hayes, D.N.; Major, M.B, *Cancer Res.* (2013) **73**, 2199–2210.
[2] Matić, S; Kekez, I.; Tomin, M. ; Bogár, F. ; Šupljika, F. ; Kazazić, S. ; Hanić, H.; Jha, S.; Brkić, H.; Bourgeois, B.; Madl, T.; Gruber, K.; Macheroux, P.; Matković-Čalogović, D.; Matovina, M.; Tomić, S., *J Biomol. Struct. Dynamics* (2020), doi: 10.1080/07391102.2020.1804455.
[3] Matić, S.; Tomašić Paić, A.; Sobočanec, S.; Pinterić, M.; Pipalović, G.; Martinčić, M.; Matovina, M.; Tomić, S., *IJMS*, 23 (2022), doi:10.3390/ijms23041994 .