Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43A, No.2,
July-December 2024: P.242-249

Print version ISSN 0970 0765
Online version ISSN 2320 3188

Original Article
Content Available online at: https://www.bpasjournals.com/

In-Silico Analysis of FANCD2 mutations presents in the FANCD2 protein MLS and their subsequent impacts

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Received on 20.06.2024
Revised on 10.10.2024
Accepted on 20.11.2024

ABSTRACT:
The Fanconi Anemia complement protein plays a significant role in fixing the damage caused by nuclear intercross links in DNA. The in-silico analysis of the Fanconi anemia complement protein D2's unique mitochondrial localization signal sequence at the N-terminal of D2, which is 30 AA long, reveals the crucial steps of this FANCD2 activation through the addition of a single ubiquitin unit. An important role of FANCD2 in mitochondria is established by the cosmic database analysis of the 30 AA residues of MLS. Different cancer types develop in response to mutations within MLS.

Keywords:
Fanconi Anemia, Mitochondria, Mitochondrial Localization Signal, Cancer, DNA Repair.

How to cite this article: Mahto R.B. (2024). In-Silico Analysis of FANCD2 mutations presents in the FANCD2 protein MLS and their subsequent impacts. *Bulletin of Pure and Applied Sciences-Zoology*, 43A (2), 242-249.

1. INTRODUCTION

When a gene is lost or changes in a way that makes it nonfunctional, FA is concerned. FA complements protein controls fixing intercross-link DNA damage. It is a rare kind of autosomal recessive genetic disease. Consequences include the manifestation of numerous uncommon symptoms during adolescence, as evidenced by the conformation of cellular ICL DNA in response to oxidative stress. The accumulation of random mutations across the entire genome results from a deficiency in the ICL repair mechanism, which is functional in normal cells. The deposition of random mutations may lead to the development of cancer in different organs. For a detailed study of how cancer develops in multiple organs, this is the most suitable model. Currently, researchers have identified 19 genes that work together to repair intercross-link DNA damage, a common goal. An interesting fact is that the FANCD2 protein functions as an effector to operate ICL DNA, which is activated by adding one unit of ubiquitin.

1. FA leads to the development of cancer in the different organs. One of the common symptoms is the development of blood cells in the bone marrow if failure/reduction forms faulty/transformed cells. In the case of Fanconi anemia, the patient's common defective organ is concerned with the actively dividing cells. The presence of actively dividing cells in the organ increases the likelihood of mutation accumulation, which in turn leads to transformations. Many organs in Fanconi anemia experience random mutations, providing scientists with an excellent opportunity to explore the true path of transformations. FA pathways prevent tumor development by repairing intercross-link DNA damage. More than 19 protein members are linked with FA pathways; eight members of the FA core complex (FANCA, FANCB, FANCC, FANCE, FANCF, FANCG, FANCL, and

FANCM) sense the ICL damage, while accessory proteins FAAP100 and FAAP24 operate in the nucleus. FA core member FANCL acts as the E3 Ubiquitin ligase (Meetei, A. R., et al., 2003), which activates the effector FANCD2-I through monoubiquitination.

2. FANCD2 and FANCI work together with nuclear protein. When DNA is damaged, FANCD2 and FANCI go through monoubiquitination at Lysine 561 and Lysine 521, respectively. A process called ubiquitination brings FANCD2-I to chromatin foci, where it joins FANCJ and FANCN. Due to the presence of repair components such as Rad51, BRCA1, BRCA2, NBS1, PCNA, or H2AX, these foci are considered DNA repair structures. We still don't know how these changes happen or what role ubiquitinated FANCD2-I plays in these foci. ATM phosphorylates FAD2 at Ser 222 in response to infrared radiation, which starts an S-phase checkpoint response (Huang, T. T., et al., 2006). These data indicate that FANCD2 possesses two distinct roles in response to DNA damage. Recent research has shown that interstrand cross-linking agents cause phosphorylation of FANCD2 and FANCI that is dependent on ATR. ATR not only affects FANCD2 and FANCI, but it also phosphorylates FANCA after DNA cross-links are made. This is an important step for FANCA to stay in the nucleus and for cross-link repair to work (Collins, N. B., et al, 2009). ATR phosphorylates FANCG to aid in repair (Qiao, F., et al., 2004). Recent investigations indicate the presence of a high molecular weight FA complex in both the nucleus and cytoplasm (Thomashevski, A., et al., 2004). This indicates that the complex may operate in multiple cellular compartments. In addition to the FANCC protein, research identifies its interactions with the molecular chaperones GRP94 and HSP70, NADPH cytochrome P450 reductase, implicated in xenobiotic biotransformation, glutathione S-transferase (GSTP1), associated with redox metabolism, and STAT1, involved in signaling (Wang, J., et al., 1998; Pang, E. S., 2000). FANCA is a phosphoprotein that interacts with the IkB kinase (IKK) signal through IKK2, thereby enhancing NF-kB signaling, which may influence apoptosis (Azuma, H., et al., 2002).

2. MATERIASLS AND METHODS

In-Silico Analysis of FANCD2 for Mitochondrial Localization Signal (MLS):

2.1 MitoProt

(*https://urgi.versailles.inra.fr/predotar/predotar.html*)

Predotar is an online tool aimed at the efficient screening of large groups of proteins for identifying signal sequences with a very low rate of false positives. Predotar recognizes the NTD signal sequences of the query proteins. This tool, Predotar, gives a rough guess for each protein sequence based on whether it has an MLS (mitochondrial localization signal) sequence, a plastid targeting sequence, or an ER targeting sequence. A targeting sequence is well indicated by a probability threshold value above 0.2. The FASTA format of the query proteins is given to the query box by selecting the source of the protein.

2.2 COSMIC v99, released 28-NOV-23:

COSMIC, the Catalogue of Somatic Mutations in Cancer, is the world's largest and most comprehensive resource for exploring the impact of somatic mutations in human cancer. The gene view histogram is a graphical view of mutations across FANCD2_ENST00000383807. The MLS 30 amino acid region of FANCD2 NTR displays these mutations at the amino acid level (https://cancer.sanger.ac.uk/cosmic/gene/analysis).

2.3 Prot pi | Peptide Tool

We use Prot pi | Peptide Tool to calculate the isoelectric point and net charge of peptides. Isoelectric point and net charge of mls strongly influence the migration of proteins. Different SNPs within MLS of FANCD2 evaluate both of these parameters.

2.4 **GOR4 secondary structure prediction method.**

Mutations in MLS cause minute changes in protein structure, which can have a significant impact on functions. Here we use the GOR4 tools to evaluate the structural change at the secondary level. The cosmic database of Fanconi patients reports structural changes due to mutation within MLS, which we are particularly interested in seeing.

3. RESULTS AND DISCUSSION

3.1 All 19 FA proteins analyzed by various in-silico tools for MLS and NLS.

We conducted a comprehensive analysis of all 19 FA proteins using various in silico tools to identify mitochondrial localization signals (MLS) and nuclear localization signals (NLS). The tables present the findings. The FANCD2 protein is known to bind directly to damage DNA, playing a crucial role in the repair process; notably, FANCD2 also possesses an MLS contributing to the repair of mitochondrial DNA damage. This computer study showed that both the FANCD2 and FANCG proteins had mitochondrial localization signals, which can be seen in tables 1 and 2. We identified a signal sequence of 30 amino acids at the N-terminal of both FANCD2 and FANCG using online tools like IPSORT. The MLS provides significant insights for subsequent analyses. Table 2 highlights the 30 amino acid sequences of the MLS in fancd2 serial number 5 and FANCG serial number 8 in green.

Table 1: In-silico characterization of FA proteins for their localization into the mitochondria by TargetP 1.1, MitoProt II – v1.101, iPSORT, Predotar, TPpRED2.0.

PROTEIN	TargetP 1.1		MitoProt II – v1.101		iPSORT		Predotar	TPpRED2.0	
	mTP	RC	probability	Charge	SP	MLS			
FANCA	0.223	3	0.0897	-15	NO	NO	0.00	NO	0.98
FANCB	0.061	1	0.0732	-05	NO	NO	0.00	NO	0.99
FANCC	0.059	2	0.0590	-15	NO	NO	0.00	NO	0.99
FANCD1	0.115	2	0.0169	-30	NO	NO	0.01	NO	0.99
FANCD2	**0.182**	**2**	**0.2296**	**-42**	**NO**	**YES**	**0.03**	**NO**	**0.99**
FANCE	0.090	1	0.0070	-20	NO	NO	0.00	NO	0.99
FANCF	0.056	2	0.1802	+06	NO	NO	0.00	NO	0.72
FANCG	**0.212**	**3**	**0.4097**	**-13**	**NO**	**YES**	**0.02**	**NO**	**0.92**
FANCI	0.054	1	0.1082	-03	NO	NO	0.00	NO	0.99
FANCJ	0.093	2	0.0758	-11	NO	NO	0.00	NO	0.98
FANCL	0.321	4	0.1797	-05	NO	NO	0.02	NO	0.85
FANCM	0.875	2	0.5774	-41	NO	NO	0.29	YES	0.97
FANCN	0.088	1	0.0090	-01	NO	NO	0.00	NO	1.00
FANCO	0.577	4	0.3127	-02	NO	NO	0.20	NO	0.89
FANCP	0.061	2	0.0081	-27	NO	NO	0.00	NO	0.93
FANCQ	0.050	3	0.0699	-07	NO	NO	0.00	NO	0.99
FANCR	0.059	1	0.0159	-13	NO	NO	0.00	NO	0.99
FANCS	0.087	2	0.0248	-86	NO	NO	0.00	NO	0.98

FANCT	0.346	4	0.2180	5	NO	NO	0.04	NO	0.98
Maximum probability of the mitochondrial localization of FA proteins is showed in **bold**. mTP: mitochondrial Targeting Peptide; RC: reliability class; SP: Signal Peptide; MLS: Mitochondrial Localization Signal.									

Table 2: Through iPSORT prediction clearly identify the 30 AAs sequence of FANCD2 and FANCG at N terminal of protein having mitochondrial localization signal.

S. No.	FANC Protein	N-Terminal sequence	MLS
1	FANCA	MSDSWVPNSASGQDPGGRRRAWAELLAGRVK	NO
2	FANCB	MTSKQAMSSNEQERLLCYNGEVLVFQLSKG	NO
3	FANCC	MAQDSVDLSCDYQFWMQKLSVWDQASTLET	NO
4	FANCD1/BRCA2	MPIGSKERPTFFEIFKTRCNKADLGPISLN	NO
5	**FANCD2**	**MVSKRRLSKSEDKESLTEDASKTRKQPLSK**	**YES**
6	FANCE	MATPDAGLPGAEGVEPAPWAQLEAPARLLL	NO
7	FANCF	MESLLQHLDRFSELLAVSSTTYVSTWDPATV	NO
8	**FANCG**	**MSRQTTSVGSSCLDLWREKNDRLVRQAKVA**	**YES**
9	FANCI	MDQKILSLAAEKTADKLQEFLQTLREGDLT	NO
10	FANCJ/BRIP1	MSSMWSEYTIGGVKIYFPYKAYPSQLAMMN	NO
11	FANCL	MAVTEASLLRQCPLLLPQNRSKTVYEGFIS	NO
12	FANCM	MSGRQRTLFQTWGSSISRSSGTPGCSSGTE	NO
13	FANCN/PALB2	MDEPPGKPLSCEEKEKLKEKLAFLKREYSK	NO
14	FANCO/RAD51C	MRGKTFRFEMQRDLVSFPLSPAVRVKLVSAG	NO
15	FANCP/SLX4	MKLSVNEAQLGFYLGSLSHLSACPGIDPRS	NO
16	FANCQ/ERCC4	MESGQPARRIAMAPLLEYERQLVLELLDTD	NO
17	FANCR/RAD51C	MAMQMQLEANADTSVEEESFGPQPISRLEQ	NO
18	FANCS/BRCA1	MDLSALRVEEVQNVINAMQKILECPICLELI	NO
19	FANCT	MQRASRLKRELHMLATEPPPGITCWQDKDQM	NO

3.2 Mutation in FANCD2 associated with disease analyzed by using database.

The FANCD2 protein demonstrates a significant degree of conservation, which reflects its critical importance. The MLS of FANCD2 is extensively conserved across a range of organisms, further supporting the assertion that FANCD2 is vital for mitochondrial functionality. Additionally, a mutation within the MLS of FAD2 lined with disease has been analyzed through various databases. The cosmic database, along with other databases created by Rockefeller University, regarding Fanconi anemia complement proteins, was employed to identify patients with mutations at the N-terminus of their FAD2, emphasizing the importance of the FAD2 MLS. The cosmic database recognized as a comprehensive mutation database for cancer reveals that mutations occurring within the MLS at many positions of FAD2 are implicated in cancers affecting various primary tissues (refer to Fig. 1 Table 3).

3.3 Change in structure, an isoelectric point and net charge looses function of FANCD2 MLS.

MLS function is very clear and plays a role in localization of protein into mitochondria. Here we find out that there is much structural change in MLS of FANCD2 reported in the cosmic database of FA patients (tables 3 and 4). We highlight the major structural change as well as change in an isoelectric point and net electric charge due to mutation. For the efficient function of MLS, the definite structure, an isoelectric point, and net electric charge are very essential. From the above observation, we understand that because of the mutation, FANCD2 is unable to target mitochondria. Because of a lack of mitochondrial localization,

FAND2 is unable to perform the usual function of mitochondrial DNA ICL damage repair. The complexity generated in the form of an increase ROS level, accumulation of mutations, and transformation.

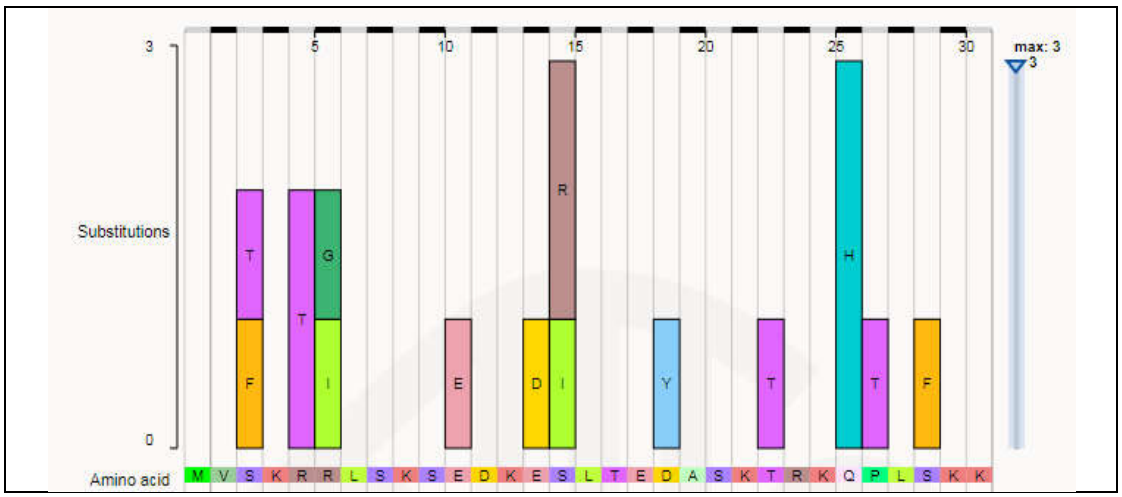

Figure 1: Mutations in MLS part of FANCD2 (Cosmic global mutation data base for cancer)

Table 3: Shows mutation at different position within 30AA residue of MLS in FANCD2.

Mutation in MLS	Genomic Mutation ID	**Primary Tissue**	**Primary Histology**	**Pubmed ID, references**
S3F	COSV107286679	Skin	Malignant melanoma	36219477, (Birkeälv, S et al., 2023).
S3T	COSV104599290	Skin	Malignant melanoma	28467829, (Hayward, N. K., et al., 2017).
R5T	COSV55036087	Urinary tract	Carcinoma	32321774, (Conway, J., et al., 2020).
R6I	COSM7946246	Skin	Malignant melanoma	-----
R6G	COSV105155845	Lung	Carcinoma	29535388, (Travis, W., et al. 2019).
E11E	COSV104599166	Prostate	Carcinoma	31874108, (Mateo, J., et al., 2020).
E14D	COSV55046760	Lung	Carcinoma	22980975, (Imielinski, M., et al., 2012).
S15I	COSM9995230	Lung	Carcinoma	32321774, (Abou Alaiwi, S., et al., 2020).
S15R	COSV105155867	Prostate	Carcinoma	32268276, (Kohli, M., et al., 2020).
D19Y	COSV55047680	Urinary tract	Carcinoma	-------
T23T	COSV55036868	Stomach	Carcinoma	--------
Q26H	COSV55036019	Haematopoietic and lymphoid Prostate	Chronic myelo monocytic leukaemia	26648538, (Mason, C. C., et al., 2016). 31874108, (Mateo, J., et al., 2020).

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P27T	COSV105156026	Pancreas	Carcinoma	26804919, (Gingras, M. C., et al., 2016).
S29F	COSV55048072	Skin	Carcinoma	25759019, (Sharpe, H. J., et al., 2015).
Many mutations in MLS part of FANCD2 associated with cancer (global mutation data base for cancer)				

Table 4: Shows secondary structural change due mutation at different position within 30AA residue of MLS in FANCD2.

Mutation in MLS	Genomic Mutation ID	Amino Acid sequence of FANCD2 MLS and corresponding secondary structure	Isoelectric point: *pI*	Net charge *z* at pH 7.4:	Primary Histology
Wild type	FANCD2 MLS	MVSKRRLSKSEDKESLTEDASKTRKQPLSK ccceeecccccchhhhhhhhhhhhcceec	9.877	+3.259	Normal
S3F	COSV107286679	MVFKRRLSKSEDKESLTEDASKTRKQPLSK ccceeecccccchhhhhhhhhhhhcceec	9.877	+3.259	Malignant melanoma
S3T	COSV104599290	MVTKRRLSKSEDKESLTEDASKTRKQPLSK ccceeecccccchhhhhhhhhhhhcceec	9.877	+3.259	Malignant melanoma
R5T	COSV55036087	MVSKTRLSKSEDKESLTEDASKTRKQPLSK Ccceeecccccchhhhhhhhhhhhcceec	9.567	+2.259	Carcinoma
R6I	COSM7946246	MVSKRILSKSEDKESLTEDASKTRKQPLSK ccceeecccccchhhhhhhhhhhhcceec	9.567	+2.259	Malignant melanoma
R6G	COSV105155845	MVSKRGLSKSEDKESLTEDASKTRKQPLSK ccceecccccccchhhhhhhhhhhhcceec	9.567	+2.259	Carcinoma
E14D	COSV55046760	MVSKRRLSKSEDKDSLTEDASKTRKQPLK cccceeecccccccchhhhhhhhhhcceec	9.877	+3.258	Carcinoma
S15I	COSM9995230	MVSKRRLSKSEDKEILTEDASKTRKQPLSK Cccceecccccchhhhhhhhhhhhcceec	9.877	+3.259	Carcinoma
S15R	COSV105155867	MVSKRRLSKSEDKERLTEDASKTRKQPLK cccceecccccchhhhhhhhhhhhcceec	10.187	+4.259	Carcinoma
D19Y	COSV55047680	MVSKRRLSKSEDKESLTEYASKTRKQPLSK Cccceeecccccchhhhhhhhhhcccceec	10.000	+4.255	Carcinoma
Q26H	COSV55036019	MVSKRRLSKSEDKESLTEDASKTRKHPLSK Cccceeecccccchhhhhhhhhhcccceec	9.877	+3.304	Chronic myelo monocytic leukaemia
P27T	COSV105156026	MVSKRRLSKSEDKESLTEDASKTRKQTLSK ccceeecccccchhhhhhhhhhhhheeec	9.877	+3.259	Carcinoma
S29F	COSV55048072	MVSKRRLSKSEDKESLTEDASKTRKQPLFK ccceeecccccchhhhhhhhhhhhcceec	9.877	+3.259	Carcinoma
Many mutations in MLS part of FANCD2 associated with cancer (global mutation data base for cancer), c= coil, h= helix,e= extended region of protein.					

3. CONCLUSIONS

The FANCD2 complement protein of the FA pathway targets mitochondria. The MLS of FANCD2 plays a crucial role in targeting the MLS of FAD2. A mutation in the MLS of FAD2 results in structural changes, an isoelectric point change, and a net electric charge change. Because of a lack of mitochondrial localization, FAND2 is unable to perform the usual function of mitochondrial DNA ICL damage repair. This complexity manifests as an increase in ROS levels, accumulation of mutations, and transformation. The cosmic database unequivocally implicates the FANCD2 MLS mutation in the development of carcinoma, chronic mylomonocytic leukemia, and malignant melanoma. Similar to the nuclear DNA damage response, FANCD2 may also play a role in repairing mitochondrial DNA damage it.

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