FANCJ regulates the stability of FANCD2/FANCI proteins and protects them from proteasome and caspase-3 dependent degradation.

Komaraiah Palle, Ph.D. (Kumar)
Assistant Professor of Oncologic Sciences
Abraham Mitchell Cancer Research Scholar
Mitchell Cancer Institute
University of South Alabama
Outline

• Fanconi anemia (FA) pathway
• Role of FA pathway in Genome maintenance
• FANCIJ and FANCD2 functional relationship
• FANCIJ-mediated DDR in response to Fork-stalling
Fanconi Anemia

- Rare, inherited blood disorder.
- 1:130,000 births
- Affects men and women equally.
- Affects all racial and ethnic groups
  - higher incidence in Ashkenazi Jews and Afrikaners
Birth Defects
Fanconi anemia pathway

- FA is a rare chromosome instability syndrome
- Autosomal recessive disorder (or X-linked)
- Developmental abnormalities
- 17 complementation groups identified to date
- FA pathway is involved in DNA repair
- Increased cancer susceptibility
  - many patients develop AML
  - in adults solid tumors
Fanconi Anemia is an aplastic anemia.
FA patients are prone to multiple types of solid tumors

- Increased incidence and earlier onset cancers: oral cavity, GI and genital and reproductive tract head and neck breast esophagus skin liver brain

Why?
FA is a DNA repair disorder

- **FA caused by mutations in 17 genes:**
  
<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Gene 2</th>
<th>Gene 3</th>
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</thead>
<tbody>
<tr>
<td>FANCA</td>
<td>FANCF</td>
<td>FANCM</td>
</tr>
<tr>
<td>FANCB</td>
<td>FANCG/XRCC9</td>
<td>FANCN/PALB2</td>
</tr>
<tr>
<td>FANCC</td>
<td>FANCI</td>
<td>RAD51C/FANCO</td>
</tr>
<tr>
<td>FANCD1/BRCA2</td>
<td>FANCJ</td>
<td>SLX4/FANCP</td>
</tr>
<tr>
<td>FANCD2</td>
<td>FANCL</td>
<td>ERCC2/XPF/FANCO</td>
</tr>
<tr>
<td>FANCE</td>
<td></td>
<td>BRCA1/FANCS</td>
</tr>
</tbody>
</table>

- **FA genes function in DNA repair processes**
- **FA patient cells are highly sensitive to DNA crosslinking agents**
Fanconi anemia is a disease of DNA repair

Core complex, consists ubiquitin ligase

Activation of FA pathway in response to replication stress/fork stalling

Repair complex

Promotes error free HR

Taniguchi’s Lab
FANCJ

- 5’ to 3’ DNA helicase in RecQ family
- Acts as a tumor suppressor
- Directly associates with BRCA1
- Also known as BRCA1-interacting protein 1 (BRIP1)
- FANCJ mutations lead to increased risk of breast and ovarian cancers
- FANCJ known to act downstream to FANCD2 in DNA repair
FANCJ interacts with numerous proteins that function in DNA damage response / repair

- Replication protein A (RPA)
- BRCA1 & 2
- BARD1
- RAD51
- MutL Homolog 1 (MLH1)
- Topoisomerase II binding protein 1 (TOPBP1)
- BLM
- FANCM
- The MRN complex – MRE11, RAD50 and NBS1
Figure 15. Map of FANCJ functional domains, protein interaction sites and mutations. This figure illustrates the various domains of FANCJ and the mutations. There are 14 clinically relevant FANCJ mutants, including 7 identified in FA, 6 in breast cancer, and 1 in both. There are 3 mutations that are known to alter helicase activity – K52R abolishes activity and P47A and M299I decrease helicase function.
G4 Resolution by FANCIJ Helicase

- Preservation of chromosomal structure
- Smooth replication
- Maintenance of genomic stability
- Transcriptional regulation

Brosh Jr. & Cantor 2014
FANCD2

- Considered the FA pathway effector protein
- Forms a complex with FANCI
- Monoubiquitinated by the FA core complex in response to DNA damage
- Activation of FANCD2 is necessary for repair of crosslinks and for homologous repair
Evidence showing FANCJ may act earlier in the pathway

FANCJ/BRIP1 recruitment and regulation of FANCD2 in DNA damage responses

Fan Zhang · Qiang Fan · Keqin Ren · Arleen D. Auerbach · Paul R. Andreassen
Environmental Genotoxins

Poly aromatic hydrocarbons (PAH)

Benzo-[a]-pyrene

Solar UV radiation

UV light

Cyclobutane pyrimidine dimers (CPDs)

Bulky DNA adducts

From DNA Repair and Mutagenesis, Friedberg, E. C. ed.
Chemotherapeutic agents

Cis-Platin

Camptothecin
Camptothecin poisoning of Top1 induces replication stress
FANCJ is important for FANCD2 foci formation in response to replication stress
FANCJ is important for FANCD2 stability in response to replication stress

siFANCJ: - - + + +
HU: - + - + *

FANCD2
FANCJ
pChk1(317)
Chk1
pChk2(T68)
pRPA32(S4/8)
β-actin
FANCJ regulates the stability of FANCD2/FNACI complex in multiple cell lines
FANCJ regulates the stability of FANCD2/FNACI complex in multiple cell lines
FANCD2 stability depends on FANCJ protein
FANCJ regulates stability of FANCD2/FNAC1 proteins
FANCD2 status affects FANCI but does not influence much on FANCJ protein.
FANCD2 status affects FANCI but does not influence much on FANCJ protein.
FANCIJ regulates the stability of FANCD2/FNACI complex
Cyclohexamide treatment reveals FANCD2 protein levels depends on FANCJ
FANCJ deficiency does not affect cell cycle profile

![Graph showing cell cycle profiles for siCon and siFANCJ conditions.](image-url)
Proteasome and Pan-caspase inhibitors rescue FANCD2 protein in the absence of FANCJ

<table>
<thead>
<tr>
<th>Treatment</th>
<th>siControl</th>
<th>siFANCJ</th>
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<tbody>
<tr>
<td>Leupeptin</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZVAD</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MG132</td>
<td>-</td>
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</tbody>
</table>

![Western Blot Image](image)

- FANCD2
- FANCJ
- GAPDH
Pan-caspase inhibitor rescue FANCD2 protein in the absence of FANCJ
FANCJ deficiency promotes polyubiquitination and caspase-dependent degradation of FANCD2
Caspase-3 downregulation in FANCJ deficient cells rescues FANCD2
FANCJ protein but not its helicase activity important for FANCD2 stability
FANCJ and FANCD2 proteins co-immunoprecipitates in the absence of DNA damage
FANCJ is important for integrity of FA pathway

FANCJ proficient

FANCJ-K52R (helicase dead)

FANCJ deficient

Cytoplasm

Nucleus

DNA repair/ fork restart

DNA repair/ fork restart

Degradation by Proteasome and Caspase-3
Summary

- FANCJ is important for the stability of FANCD2/FANCI complex

- FANCJ is necessary for proper activation of DNA damage response

- FANCJ protects FANCD2/FANCI from degradation by the ubiquitin proteasome and caspase-3 dependent mechanisms

- FANCJ is the key regulator of DDR to replication inhibitors

- FANCJ has both helicase dependent and independent functions in DDR
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