Posttranslational Regulation of the Fanconi Anemia Cancer Susceptibility Pathway

Assistant professor
Department of Pharmacological Sciences
Stony Brook University

Hyungjin Kim, Ph.D.
DNA Repair System Preserves Genome Stability

**DNA DAMAGE**

- X-rays
- Oxygen radicals
- Alkylating agents
- Spontaneous reactions
- UV light
- Polycyclic aromatic hydrocarbons
- X-rays
- Anti-tumor agents (cisplatin, MMC)
- Replication errors

- Uracil
- Abasic site
- 8-Oxoguanine
- Single-strand break
- (6-4)PP
- Bulky adduct
- CPD
- Interstrand cross-link
- Double-strand break
- Base pair mismatch
- Insertion/deletion

**DNA REPAIR**

- Base-excision repair (BER)
- Nucleotide-excision repair (NER)
- Fanconi anemia (FA) pathway
- HR/NHEJ
- Mismatch repair

**DNA DAMAGE RESPONSE**

- Cell-cycle arrest
- Cell death

**DNA REPAIR DEFECT**

- Cancer
  - Mutations
  - Chromosome aberrations

Adapted from Hoeijmakers (2001) Nature
DNA Repair Mechanism as a Tumor Suppressor Network

Cancer is a disease of DNA repair

Environmental Mutagen
Oncogenic Stress

DNA damage response

Checkpoint, DNA repair

Tumorigenesis

Genome instability

- Hereditary cancers: mutations in DNA repair genes (BRCA1, BRCA2, MSH2, etc)

- Sporadic cancers:
  - Oncogene-induced replication stress
  - Inaccurate DNA repair caused by selection of somatic mutations in DNA repair genes
Germ-line and Somatic Disruption of DNA Repair Is Prevalent in Cancer

The Cancer Genome Atlas (TCGA):
Genomic characterization and sequence analysis of various tumors from patient samples

Extent of HR Defects in TCGA Ovarian Samples

- BRCA1
- BRCA2
- C11orf30
- PTEN
- RAD51C
- ATM
- ATR
- PALB2
- FANCA
- FANCC
- FANCI
- FANCL
- FANCD2
- FANCE
- FANCG
- FANCM

- 20% - BRCA1/2 Somatic and Germline Mutations
- 31% - with BRCA1 Epigenetic Silencing
- 37% - with C11orf30 / EMSY Alterations
- 42% - with PTEN Alterations
- 49% - with RAD51C, ATM, ATR and Fanconi Anemia Genes

TCGA network, (2011) Nature
Clinical and Cellular Features of Fanconi Anemia (FA)

Fanconi Anemia

Chromosome instability syndrome

Mutation in 17 genes that cooperate in DNA interstrand cross-link (ICL) repair i.e. the FA pathway

Disease of defective DNA repair
Model for cancer pathogenesis

Developmental defect
Bone marrow failure
Increased cancer risks
The Seventeen Fanconi Anemia Genes in the FA/BRCA Pathway

17 FANC genes
(Complementation group)

- FANCA
- FANCB
- FANCC
- FANCD1
- FANCD2
- FANCE
- FANCF
- FANCG
- FANCI
- FANCJ
- FANCL
- FANCM
- FANCN
- FANCO
- FANCP
- FANCQ
- FANCS

Frequencies of FA complementation group mutations

- BRIP1/FANCJ
- FANCM
- FANCL
- FANCF
- FANCE
- FANCD2
- BRCA2/FANCD1
- FANCC
- FANCB
- XPF/FANCQ
- SLX4/FANCP
- PALB2/FANCN
- RAD51C/FANCO
- BRCA1/FANCS

60-70%
The FA Pathway Regulates Interstrand Cross-link (ICL) Repair

**FA core**

**Nucleolytic Incision**

**Translesion DNA synthesis (TLS)**

**HR**

**NER**

*Kim & D’Andrea (2012) Genes & Dev*
Regulation of FANCD2 Mono- and De-Ubiquitination

FANCD2-L: FANCD2-Ub

USP1: Deubiquitinating enzyme

Fanconi Anemia

Ubiquitin Signaling

DNA Repair Process

Cancer Pathogenesis
Bioinformatic Search of UBZ4-Containing Proteins

UBD: Ubiquitin Binding Domain

UBZ4 domain from TLS polymerase κ
QILTCPVCFAQGCISLEALNKHVEDCLDGPS

Hidden Markov Model (HMM)

Predominantly found in DNA repair factors

UBD:
Ubiquitin Binding Domain

UBZ4 (Ub-Binding Zn-finger 4)
FAAP20 Regulates FANCD2 Activation in the FA Pathway

**FAAP20 (C1orf86):** Fanconi anemia-associated protein 20 kD

FAAP20 is involved in the FA pathway, with interactions and regulatory mechanisms involving Ub, D2, and E3 ligase. The diagram illustrates the regulation of FANCD2 activation through siRNA knockdown experiments, showing a decrease in survival rates with increased MMC concentrations in cells treated with siC1orf86 compared to control and siControl conditions.
The FAAP20-FANCA Interaction Is Required for Maintaining FANCA Level

![Diagram showing the interaction between Flag-FAAP20 and FANCA](image)

**siRNA FAAP20 + siRNA resistant cDNA**

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<td>Flag-FAAP20 ΔN</td>
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</table>

**Flag-FAAP20**

- wt
- ΔC
- ΔN

**UBZ4**

**FANCA interaction**

**FANCA**
FAAP20 Connects the FA Pathway with Translesion DNA Synthesis


FA Core complex
FAAP20
D2 Monoubiquitination
UBZ4 (C)
N
Rev1 Recruitment
D2 Monoubiquitination
Nucleolytic Incision
Replication-associated HR
Nucleotide adduct bypass by TLS
ICL Recognition
ICL
FA Core complex
FAAP20

1) Nucleolytic Incision
2) TLS
3) HR
Solution Structure of the FAAP20-Ubiquitin Complex

The disordered C-terminal tail of FAAP20 is involved in ubiquitin binding

The Very C-terminal Tryptophan Is Required for Ubiquitin Binding

Isothermal Titration Calorimetry (ITC)

A  ubiquitin WT + FAAP20 WT

\[ K_d = 9.26 \, \mu M \]

\[ N = 1.07 \]

B  ubiquitin WT + FAAP20 W180A

No detectable binding
The Very C-terminal Tryptophan Is Required for DNA ICL Repair

CA: C147A & C150A
WA: W180A

Human 140 GAAALRSCICMKFAPRTQDLVWISHAQLAESTELVTW 180

* CA: C147A & C150A
* WA: W180A

% Survival vs MMC (nM)
Ubiquitin Recognition of FAAP20 Is Distinct from Canonical UBZ Module

Unconventional FAAP20 UBZ motif
Regulation of the FA Pathway by the FANCA-FAAP20 Axis

- Mechanism of maintaining FANCA stability by FAAP20?

- Mechanism of FANCA degradation and its implication in tumorigenesis?
Characterization of a FA-like Breast Cancer Patient

- 33 year-old triple negative breast cancer (TNBC) patient (not a FA patient *per se*)
- Features of FA (i.e. short stature, café au la spot, mild macrocytic anemia)

[Diagram showing a family tree with DF2231 indicated and a quadriradial chromosome marked with 20 ng/mL MMC.]
Characterization of a FA-like Breast Cancer Patient

![Graph showing survival rates with MMC dosage for different cell lines: GM0637, FA-I, and DF2231.]

Upstream defect

![Images showing FANCD2 expression with and without DAPI in GM0637 and DF2231 cells treated with MMC.]

MMC
The I939S Point Mutation in Patient FANCA Destabilizes FANCA

**FANCA gene**

2816T>G; Ile939Ser

2840C>G; Ser947stop

The Amino Acid 913-1095 Region of FANCA Is Required for FAAP20 Interaction
FANCA-I939S Mutation Disrupts FAAP20 Binding

**Diagram:**
- **mhc-FANCA**
  - WT-FANCA
  - WT-FANCA
  - I939S-FANCA

**Flag-FAAP20**
- WT-FANCA
- WT-FANCA
- I939S-FANCA

**Myc**
- WCE

**Flag**
- WCE
- Flag IP

**Experiment:**
- Input
- GST alone
- GST FANCA-WT
- GST FANCA-I939S

**Results:**
- HA-FAAP20
- GST-FANCA
- GST
Identification of SUMOylation Site of FANCA Near the FAAP20 Binding Region

SUMO consensus: ψKXE

Exon 28

Human: LSEEDVASLSWRPLHLPSADWQRAALSILWTHRTFREVLYKKEEDVHLTYQDWLHLELEIQPEADALSDTER
Mouse: CAPEHAACVPWPRLYLPSADWQRAALSILWRRDSFQELLDKEFYLTYRDWVQLELEIQPEADVLSDMER
Rat: CSEPHTARIPWKLILPSADWPRAALSILWRWSSFQELLKEEEFHLYRDWIQLELEIQPEADALSGTER
Chicken: TSKEDKADLVWSSLTCPSSLNYRASLCLWKQARFQELLKEKAFQLSFREWLLLLEEMYPEKDVLASER

Exon 29

K921, I939
Regulation of Protein Stability by Coupled SUMO-Ubiquitin Signaling

1) RNF4 is a Ubiquitin E3 ligase which regulates the DNA damage response (Galanty, Y et al, Genes Dev 26, 2012; Yin, Y, et al, Genes Dev 26, 2012)

2) RNF4 is the human ortholog of the yeast proteins, Slx5 and Slx8 (discovered in the same screen which identified Slx4; SLX4/FANCP)

**STUbL** (SUMO-Targeted Ubiquitin Ligase)

SIM: SUMO-interacting motif
FANCA from the Patient Shows Increased SUMOylation and Polyubiquitination
FANCA SUMOylation at K921 Is Required for Polyubiquitination of FANCA
UBC9 and PIAS1 Are Required for FANCA Sumoylation

[Image: Western blot analysis showing the expression levels of Myc-FANCA, PIAS1, PIAS4, UBC9, HA-SUMO3, and Tubulin under different conditions (FANCA, siControl, siUBC9, siPIAS1, and siPIAS4). The blot is probed with anti-HA IP antibodies to detect Myc-FANCA.]
FANCA-K921R SUMO-Defective Mutant Exhibits Increased Half-life

Cycloheximide blocking

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Blot: FANCA 6914 FANCA (-/-) Tubulin
RNF4 Regulates FANCA Stability

FANCA I939S + siRNF4
RNF4 Is a New Player in the FA Pathway

![Graph showing relative survival and aberrations/cell under MMC treatment]
Regulation of DNA ICL Repair by Integrated Ubiquitin-SUMO Signaling

- FANCD2 deubiquitination (USP1)
- FANCM release
- FANCA degradation
Regulation of DNA ICL Repair by Integrated Ubiquitin-SUMO Signaling

- RNF4 loss
- Aberrant FANCA accumulation
- Delay of replication restart
The FANC Proteins Are Predicted to be Extensively SUMOylated

<table>
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<tr>
<th>Fanconi Anemia Gene</th>
<th>Consense SUMO Conjugation sites</th>
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<tr>
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Mutational Hotspot of FANCA Corresponds to the FAAP20 Interacting Region

FANCA gene mutations from 97 FA patients

221 FANCA mutations from 89 studies including TCGA

Levran et al., (1997) PNAS

cBioPortal for Cancer Genomics
FANCA Mutations From FA and/or Cancer Patients Disrupt FAAP20 Interaction

FANCA null FA patient cells + FANCA variants
Conclusions

1. DNA repair mechanisms preserve genome integrity and their deregulation contributes to tumorigenesis

2. The FA pathway removes DNA ICL encountered during S phase, and its defect leads to cancer-prone disease, Fanconi anemia

3. Complex interplay of post-translational modification network including ubiquitination/SUMOylation controls DNA repair pathways
   - FAAP20 ubiquitin binding in regulating ICL repair
   - FANCA degradation by Ubiquitin plus SUMO

4. Compound heterozygosity of FANCA leads to (or contribute to) TNBC
   - Disruption of the DNA repair activity (germ-line or somatic) could be a major driving force for tumorigenesis
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