Phosphorylation of xeroderma pigmentosum group C regulates ultraviolet-induced DNA damage repair

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SUPPLEMENTARY METHODS

Plasmids and site-directed mutagenesis

The following primers were used to generate the mutations: S61A sense 5'-TGAACCCCATGAGCGCGCTCTTTTCTC-3' and S61A antisense 5'-GAGGAAAAAGGCTGCTGCTCATCTTTGGGGTTCA-3', S94A sense 5'-AAAGGATGAAGCCCTGCGATGGGGATGACCTC-3' and S94A antisense 5'-GAGGTCACTCCCATTGCGAGGGCTTCATCTCTTC-3', T169A sense 5'-CTGCTCTGCGCTCAATCTCTGACTCCACTTG-3' and T169A antisense 5'-CCAGTGAGTAGAGATTGAAGCCAGAGCAG-3', S399A sense 5'-GCAAGCCCCTCGGAGGAGGAAGATGAGG-3' and S399A antisense 5'-CCTCATCTTCGCGGCCAGGGCTTCGCTCCG-3', S884A sense 5'-CCTCCTGAGGGCGGCTTGCCTCCG-3' and S884A antisense 5'-GCTTCTGAGGGCGGCTTGCCTCCG-3', S883A sense 5'-CCTCCTGAGGGCGGCTTGCCTCCG-3' and S883A antisense 5'-CCTCCTGAGGGCGGCTTGCCTCCG-3', S892A sense 5'-GCCGCTTCTGCTTGAGCGCTGGTCCCCTC-3' and S892A antisense 5'-GCCGCTTCTGCTTGAGCGCTGGTCCCCTC-3', S892D sense 5'-GCCGCTTCTGCTTGAGCGCTGGTCCCCTC-3' and S892D antisense 5'-GCCGCTTCTGCTTGAGCGCTGGTCCCCTC-3'.

SUPPLEMENTARY FIGURES LEGENDS

Supplementary Figure S1. Role of XPC phosphorylation at S61, T169, S397, S399, S883, and S884 in CPD repair. (A) Immunoblot analysis of XPC and GAPDH in XPCNull cells expressing pLenti vector or pLenti-XPC (WT or Ser/Thr→ Ala mutant) constructs. (B) Slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm²) in XPCNull cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. (C) Quantification of percentage (%) of CPD repair from (B). *, P < 0.05,
compared with vector, Student’s t-test. (D) Slot blot analysis of the levels of CPD in sham control and UVB (20 mJ/cm²) irradiated XPCNull cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. (E-J) Slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm²) in XPCNull cells expressing pLenti-XPC WT and mutant constructs S397A (E), S399A (F), T169A (G), S883A (H), S884A (I), S61A (J). Methylene blue staining was used for loading control. The results were obtained from three independent experiments.

**Supplementary Figure S2.** Role of XPC phosphorylation at S61, T169, S397, S399, S883, and S884 in 6-4PP repair. (A) Slot blot analysis of the levels of 6-4PP at the indicated times post-UVB (20 mJ/cm²) in XPCNull cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. (B) Quantification of percentage (%) of 6-4PP-repair from (A). ***, P ≤ 0.001, compared with vector, Student’s t-test. (C) Slot blot analysis of the levels of 6-4PP in sham control and UVB (20 mJ/cm²) irradiated XPCNull cells expressing pLenti vector or pLenti-WT XPC. Methylene blue staining was used for loading control. (D-H) Slot blot analysis of the levels of 6-4PP at the indicated times post-UVB (20 mJ/cm²) in XPCNull cells expressing pLenti-XPC WT and mutant constructs T169A (D), S397A (E), S883A or S399A (F), S884A (G), S61A (H). Methylene blue staining was used for loading control. The results were obtained from three independent experiments.

**Supplementary Figure S3.** Effect of pharmacological CK2 inhibition on CPD repair and UVB regulation of CK2 levels. (A) HaCaT cells were pretreated with vehicle or CK2 inhibitor CX-4945 (5µM) for 1 h, exposed to UVB (20 mJ/cm²), and incubated for 30 min. The levels of XPC and GAPDH were analyzed by immunoblot assay. (B) Slot blot analysis of the levels of CPD at the indicated times post-UVB (20 mJ/cm²) in HaCaT cells pretreated with vehicle or CX-4945 (5 µM) for 1 h. Methylene blue staining was used for loading control. (C) Quantification of percentage (%) of CPD repair from (B). *, P < 0.05; ***, P ≤ 0.001; compared with the vehicle group, Student’s t-test. (D) Immunoblot analysis of CK2A1, CK2A2, CK2B and GAPDH in HaCaT cells 30 min after UVB exposure (20 mJ/cm²). The results were obtained from three independent experiments.
Shah et al. Supplementary Figure S1

A

XPC

GAPDH

Vector

WT XPC

S61A

S94A

T169A

S397A

S399A

S883A

S884A

B

CPD

Methylene blue

Vector

WT XPC

0 h

6 h

24 h

C

% CPD Repair

0 h

6 h

12 h

18 h

24 h

D

CPD

Methylene blue

Vector

WT XPC

Sham

UVB

E

CPD

Methylene blue

WT

S397A

0 h

6 h

24 h

F

CPD

Methylene blue

WT

S399A

0 h

6 h

24 h

G

CPD

Methylene blue

WT

T169A

0 h

6 h

24 h

H

CPD

Methylene blue

WT

S883A

0 h

6 h

24 h

I

CPD

Methylene blue

WT

S884A

0 h

6 h

24 h

J

CPD

Methylene blue

WT

S61A

0 h

6 h

24 h
Shah et al. Supplementary Figure S2

A

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B

% 6-4PP Repair

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C

Sham UVB

Vector WT XPC

Methylene blue

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E

6-4PP

Methylene blue

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H

6-4PP

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Methylene blue

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Shah et al. Supplementary Figure S3

A. Western blots showing XPC (Short Exp), XPC (Long Exp), and GAPDH expressions under UV exposure conditions.

B. Western blots showing CPD repair levels over time (0 h, 6 h, 24 h) with Vehicle and CX-4945 treatments.

C. Graph illustrating % CPD Repair over Hours Post-UVB exposure with Vehicle and CX-4945 treatments.

D. Western blots showing CK2A1, CK2A2, CK2B, and GAPDH expressions under UV exposure conditions.