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Supplemental Information

Radiation-Induced DNMT3B Promotes

Radioresistance in Nasopharyngeal Carcinoma

through Methylation of p53 and p21

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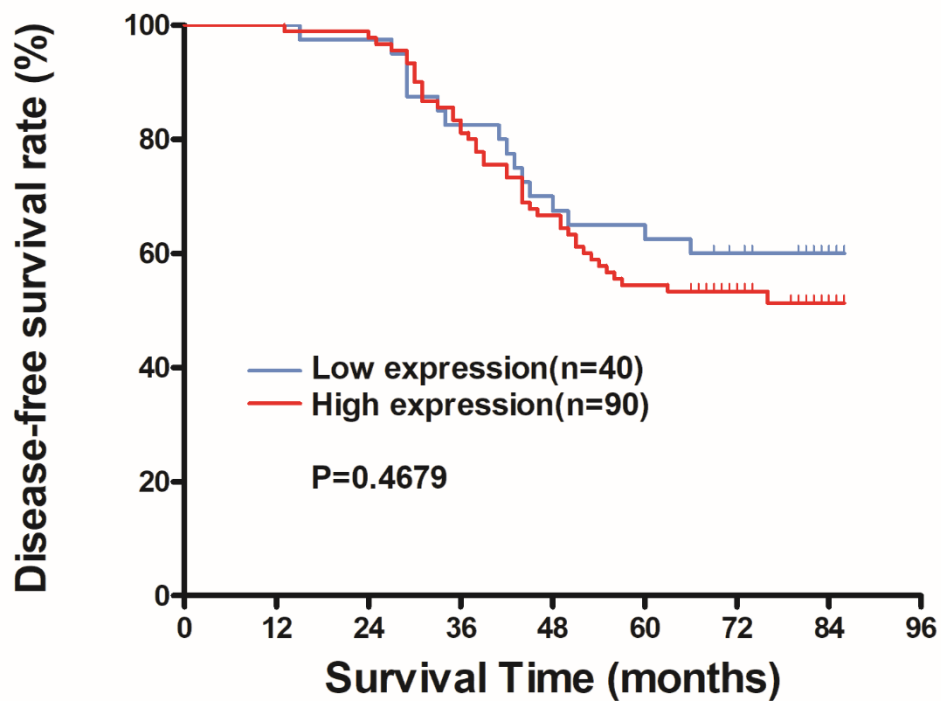


Fig. S1 DNMT3B expression is not related to disease-free survival in NPC patients. Kaplan-Meier disease-free survival curves for all 130 patients with NPC stratified by high (n=90) versus low (n=40) expression level of DNMT3B. P-value was calculated using the log-rank test.

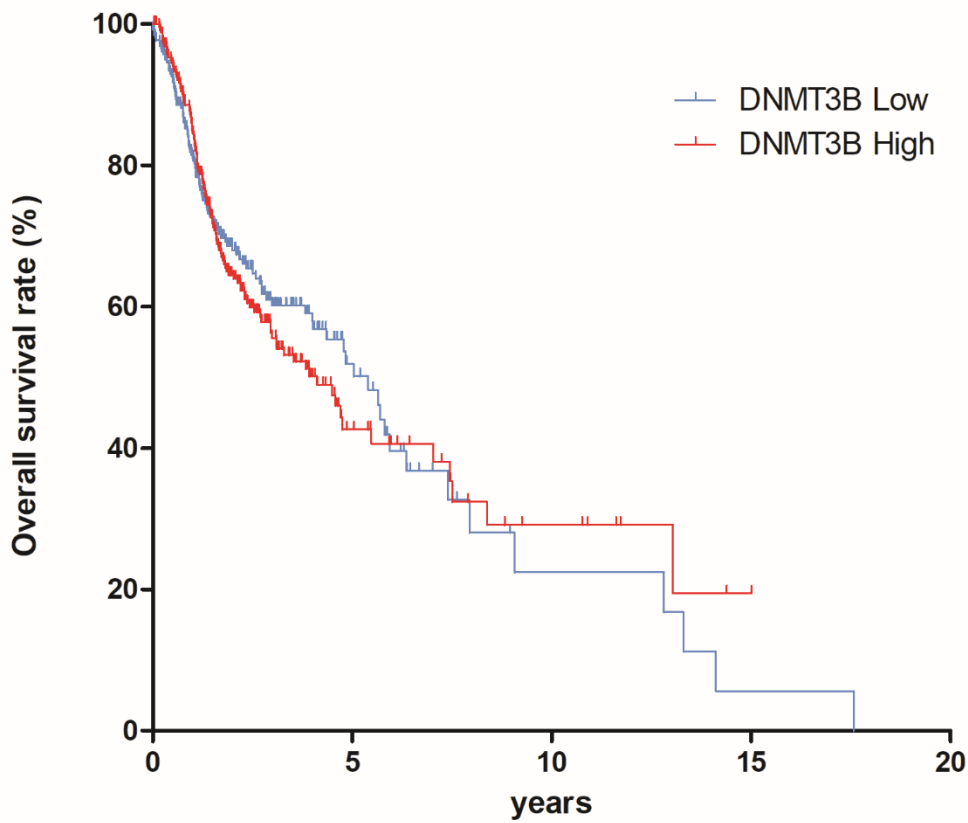


Fig. S2 DNMT3B expression is not related to overall survival in HNSCC patients from TCGA database (P=0.7246). Kaplan-Meier overall survival curves for all 518 patients with HNSCC stratified by high versus low expression level of DNMT3B. P-value was calculated using the log-rank test.

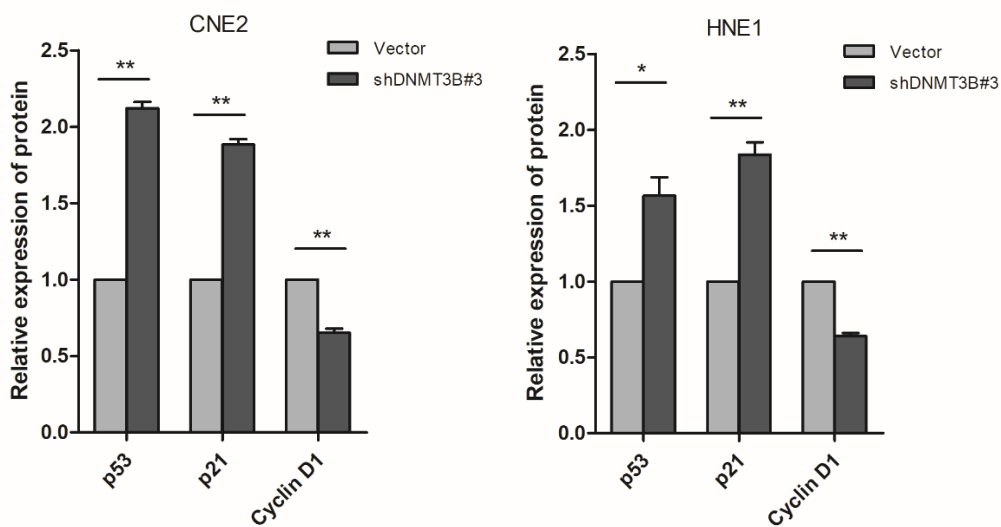


Fig.S3 Protein quantification for p53, p21 and Cyclin D1 in CNE2 and HNE1 cell lines transfected with shDNMT3B and vector.

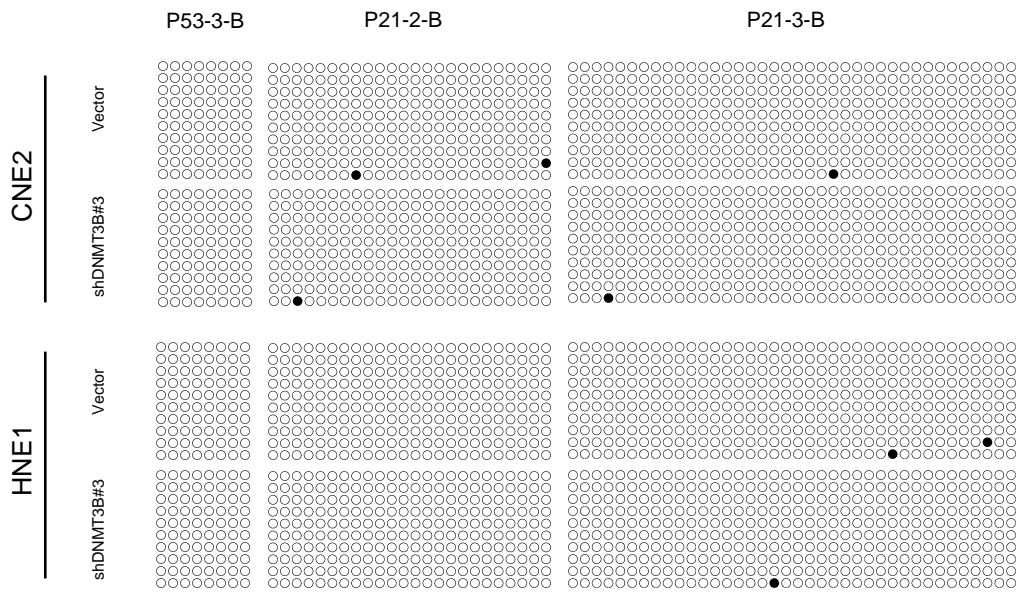


Fig.S4 Bisulfite sequencing PCR results of promoter of p53 and p21 with primers (P53-3-B, P21-2-B, P21-3-B).

Table S1. 40 paired samples with detailed information from TCGA (HNSCC).

Serial number	bcr_patient_bar code	clinical_stage	form_completion_date	anatomic_neoplasm_subdivision	gender
1	TCGA-CV-7091	Stage I	2011/10/11	Oral Cavity	MALE
2	TCGA-CV-7177	Stage I	2011/10/11	Larynx	FEMALE
3	TCGA-CV-6938	Stage II	2011/9/13	Oral Cavity	MALE
4	TCGA-CV-6961	Stage II	2011/9/12	Oral Tongue	MALE
5	TCGA-CV-6955	Stage II	2011/10/11	Oral Cavity	FEMALE
6	TCGA-CV-7101	Stage II	2011/10/6	Larynx	MALE
7	TCGA-CV-7103	Stage II	2011/10/11	Oral Tongue	MALE
8	TCGA-CV-7183	Stage II	2011/10/11	Oral Cavity	MALE
9	TCGA-CV-7238	Stage II	2011/12/5	Oral Tongue	FEMALE
10	TCGA-CV-7255	Stage II	2011/12/5	Oral Tongue	FEMALE
11	TCGA-CV-7406	Stage II	2012/4/9	Base of tongue	MALE
12	TCGA-CV-7423	Stage II	2012/2/13	Oral Cavity	MALE
13	TCGA-CV-7437	Stage II	2012/4/9	Larynx	MALE
14	TCGA-CV-7438	Stage II	2012/2/13	Oral Tongue	FEMALE
15	TCGA-CV-7440	Stage II	2012/4/9	Larynx	MALE
16	TCGA-CV-6935	Stage III	2011/9/9	Larynx	MALE
17	TCGA-CV-6943	Stage III	2011/9/9	Base of tongue	MALE
18	TCGA-CV-6959	Stage III	2011/9/8	Oral Tongue	MALE
19	TCGA-CV-6962	Stage III	2011/8/25	Larynx	MALE
20	TCGA-CV-6960	Stage III	2011/9/8	Oral Cavity	MALE
21	TCGA-CV-7097	Stage III	2011/10/5	Oral Cavity	MALE
22	TCGA-CV-7235	Stage III	2011/12/12	Floor of mouth	MALE
23	TCGA-CV-7242	Stage III	2011/12/2	Larynx	FEMALE
24	TCGA-CV-7245	Stage III	2011/12/2	Larynx	MALE
25	TCGA-CV-7250	Stage III	2011/12/5	Larynx	MALE
26	TCGA-CV-7252	Stage III	2011/12/2	Oral Cavity	FEMALE
27	TCGA-CV-7261	Stage III	2011/12/2	Larynx	MALE
28	TCGA-CV-7425	Stage III	2012/2/13	Oral Cavity	FEMALE
29	TCGA-CV-7432	Stage III	2012/2/13	Oral Cavity	MALE
30	TCGA-CV-7434	Stage III	2012/2/13	Oral Cavity	MALE
31	TCGA-HD-8635	Stage III	2012/12/28	Oral Tongue	FEMALE
32	TCGA-HD-A6I0	Stage III	2013/9/20	Oral Cavity	MALE
33	TCGA-CV-6933	Stage IVA	2011/9/9	Oral Tongue	MALE
34	TCGA-CV-6934	Stage IVA	2011/9/13	Oral Tongue	FEMALE
35	TCGA-CV-6936	Stage IVA	2011/9/13	Floor of mouth	MALE
36	TCGA-CV-6939	Stage IVA	2011/9/13	Oral Tongue	MALE
37	TCGA-CV-6956	Stage IVA	2011/10/11	Oral Tongue	MALE
38	TCGA-CV-7178	Stage IVA	2011/10/11	Oral Cavity	FEMALE
39	TCGA-CV-7416	Stage IVA	2012/1/31	Oral Cavity	FEMALE
40	TCGA-CV-7424	Stage IVA	2012/2/7	Larynx	MALE

Table S2. Methylation-specific PCR primers designed for P53 and P21.

P53-1-M-F	ATTATAGGCGCGTGTATTATATGC
P53-1-M-R	AAATCACTTAAAACCAAAAATTCTGA
P53-1-U-F	TATAGGTGTGTGTTATTATATGTGT
P53-1-U-R	AATCACTTAAAACCAAAAATTCAAA
P53-2-M-F	TTCGGTAGGCGGATTATTTG
P53-2-M-R	AAATATCCCCGAAACCCAAC
P53-2-U-F	TTGGTAGGTGGATTATTTGTTT
P53-2-U-R	CCAATCCAAAAAACATATCAC
P53-3-M-F	TTCGGTTTCGTGTATTTTAGTTC
P53-3-M-R	ACCTAAACGTTCAACTTTAAATTCG
P53-3-U-F	GTTTTTGGTTTTGTGTATTTTAGTTT
P53-3-U-R	CTACCTAAACATTCAACTTTAAATTCAA
P53-4-M-F	TTTAGTATCGCGGGTCGTTAC
P53-4-M-R	AAAATTACCGCGAAACTCGATA
P53-4-U-F	TTTTTTAGTATTGTGGGTTGTAT
P53-4-U-R	AAAATTACCACAAAACCTCAATAAAA
P21-1-M-F	TTTTTTATTGTAGGGGTAGTTACGT
P21-1-M-R	CTAAAATAATCCTACAAAATTTCGCT
P21-1-U-F	TTTTTTATTGTAGGGGTAGTTATGT
P21-1-U-R	CTAAAATAATCCTACAAAATTCACT
P21-2-M-F	TACGCGAGGTTTCGGGATC
P21-2-M-R	CCCTAATATACAACCGCCCCG
P21-2-U-F	GGATTGGTTGGTTTGTGGAATTT
P21-2-U-R	ACAACCCTAATATACAACCACCCCA
P21-3-M-F	GTGTTTTTGC GTGTTTCGC
P21-3-M-R	CGTATACGCAAACCGAACG
P21-3-U-F	GTGTGTTTTTGTGTGTTTGT
P21-3-U-R	ACCATATACACAAACCAAACA
P21-4-M-F	CGGATTCGTCGAGGTATC
P21-4-M-R	ACGCGAACACGCTTAACT
P21-4-U-F	GTGGATTTGTTGAGGTATT
P21-4-U-R	CACACAAACACACTTAACT

Table S3. Bisulfite sequencing PCR primers designed for P53 and P21.

P53-1-B-F	TTAGTTATATAAGTAGTTGGGATTATAGG
P53-1-B-R	CAAAAAAACCCTACATAATAACTCAC
P53-2-B-F	TTTAATTTTATTTTTTTTGTTTTTTT
P53-2-B-R	AAACTTACCCAATCCAAAAAAC
P53-3-B-F	TGAATTTGATGAGTTTTTTTTGAG
P53-3-B-R	AAACTCCACTCCTCTACCTAAAC
P53-4-B-F	TTTTTTGAAAGTATTGTGTTTTTTAG
P53-4-B-R	AAAACRATACAAATACTTTAAAAATTACC
P21-1-B-F	ATGGTTGTTATGAGTGTGGTTG
P21-1-B-R	CTAAAACATAAATAATCCTACAAAATTC
P21-2-B-F	GGAGGGAAGTGTTTTTTTGTAGTA
P21-2-B-R	CTTCRACAACACTCACACCTCAAC
P21-3-B-F	TAGTTTTTTGTGGAGTYGGAGTTG
P21-3-B-R	CCAAAATTCCTATACTTATAATCCC

Table S4. The primer sequences for real-time PCR analysis.

GAPDH-F	GGTCGGAGTCAACGGGTGAG
GAPDH-R	GGAAGATGGTGATGGGATTTC
DNMT1-F	CCTAGCCCCAGGATTACAAGG
DNMT1-R	ACTCATCCGATTTGGCTCTTTC
DNMT3A-F	CCGATGCTGGGGACAAGAAT
DNMT3A-R	CCCGTCATCCACCAAGACAC
DNMT3B-F	AGGGAAGACTCGATCCTCGTC
DNMT3B-R	GTGTGTAGCTTAGCAGACTGG
P53-F	CAGCACATGACGGAGGTTGT
P53-R	TCATCCAAATACTCCACACGC
P21-F	CGATGGAACTTCGACTTTGTCA
P21-R	GCACAAGGGTACAAGACAGTG

Table S5. Univariate and multivariate analysis of clinicopathological parameters for the prediction of overall survival in patients with nasopharyngeal carcinoma (n=132).

Feature	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P-value	HR (95%CI)	P-value
Age(years)				
< 45	1(Referent)			
≥45	1.349(0.5799-3.139)	0.4869		
Gender				
Male	1(Referent)			
Female	1.066 (0.3857-2.947)	0.9016		
Histologic classification				
U	1(Referent)			
D	0.4643(0.1357-1.589)	0.2215		
N category				
N ₀	1(Referent)			
N ₁₋₃	3.236(1.277-8.202)	0.0133		
Metastasis				
No	1(Referent)			
Yes	885.6(232.4-3376)	<0.0001	28.703(10.515-78.349)	<0.0001
Clinical stage				

I+II	1(Referent)	
III+IV	7.497(3.209-17.51)	<0.0001
DNMT3B expression		
Low	1(Referent)	
High	2.887(1.178-7.076)	0.0205

Abbreviations: NPC, nasopharyngeal carcinoma; U, undifferentiated non-keratinized carcinoma; D, differentiated non-keratinized carcinoma; HR, hazard ratio; 95% CI, 95% confidence interval.