第六屆 台灣藥學聯合學術研討會

2024. 11.17

New mechanisms for anti-cancer drug discovery:

LincRNA-p21 is an RNA-based DDB2 degrader as a new solution for improving cancer therapies

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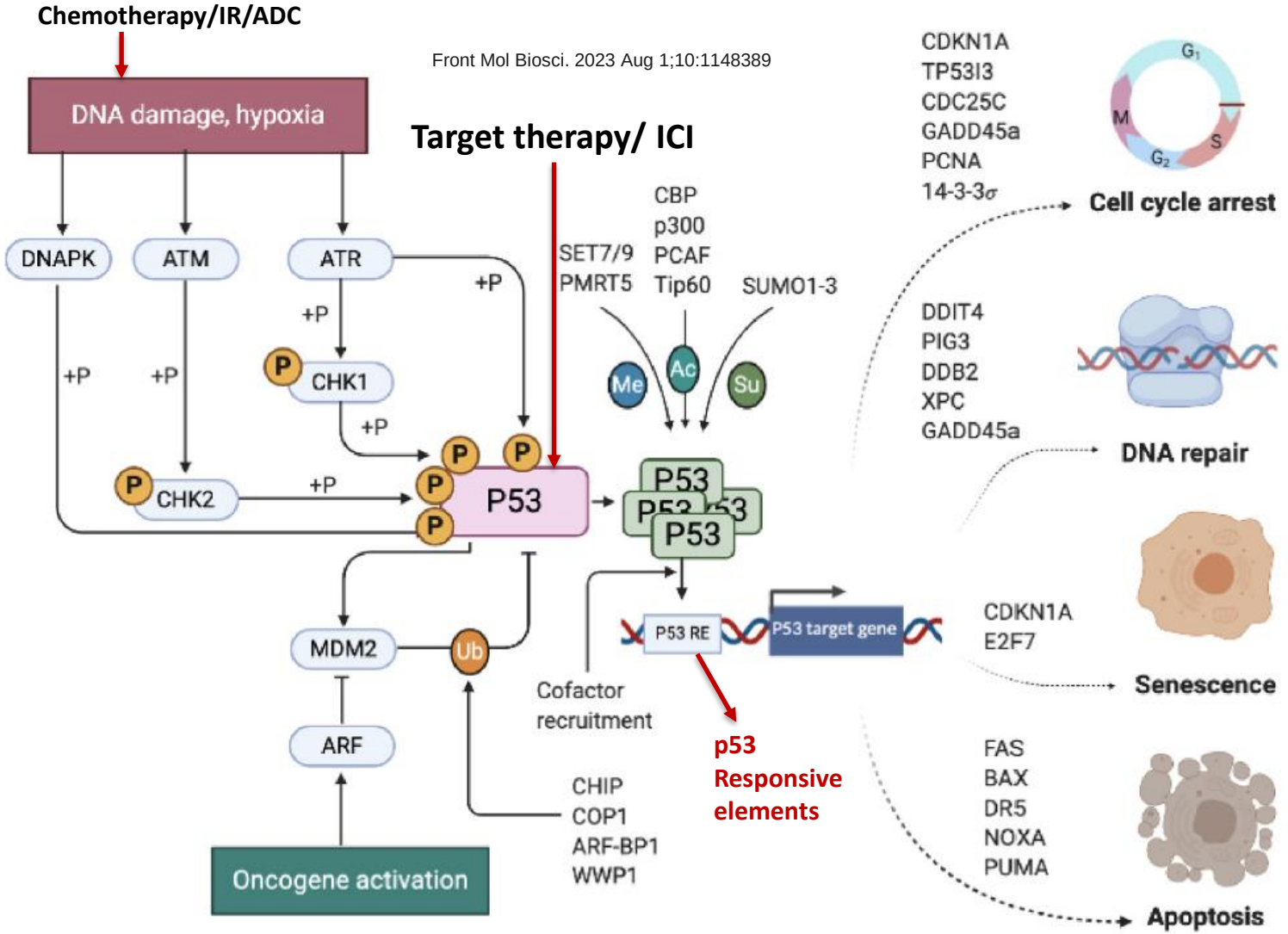
Center for Molecular Medicine, CMUH

The response rate to cancer therapies in breast cancer patients

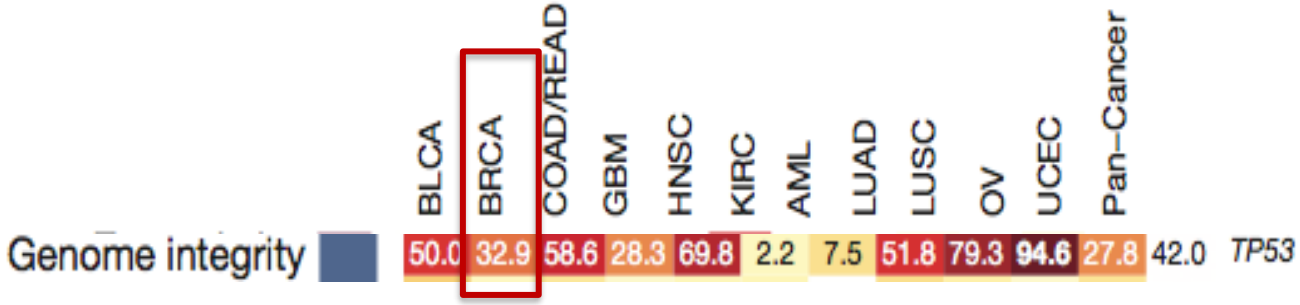
50%	80-90%	50%	20-40%	40%
Chemotherapy	**Target therapy (Specific gene)**	**Antibody-drug conjugate (ADC) (Combined treatment)**	**Immune therapy (Combined treatment)**	**Radiotherapy (R.T + Chemo)**
All subtypes	Primary HER2+	Metastatic HER2+	Metastatic TNBC	Metastatic TNBC

p53 is critical for treatment-induced cell death of tumors

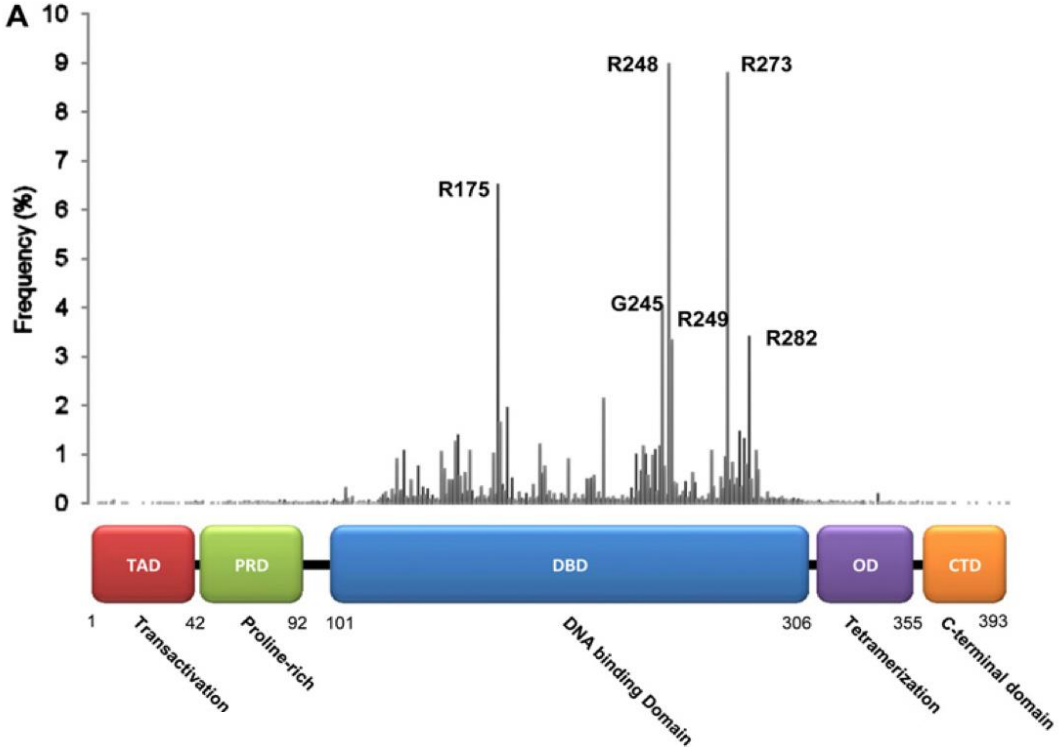


BBA-Reviews on Cancer 1876, Issue 1, August 2021, 188556

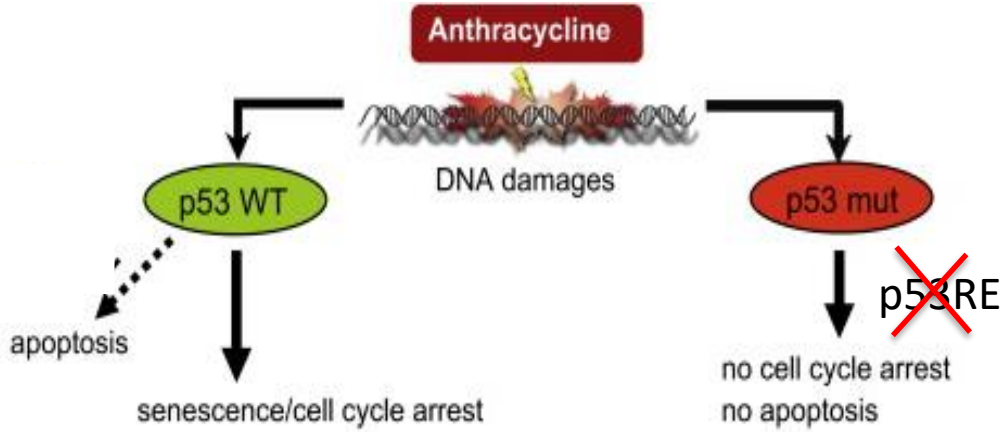
TP53 mutational spectrum and chemoresistance in human cancers



Nature. 502(7471):333-9, 2013



Genes Dev. 26(12):1268-86, 2012



Breast. 2013 Aug;22 Suppl 2:S27-9

TP53 mutation was not absolutely predictive of preferential response to chemotherapy

Neoadjuvant docetaxel plus capecitabine±trastuzumab in early-stage BC

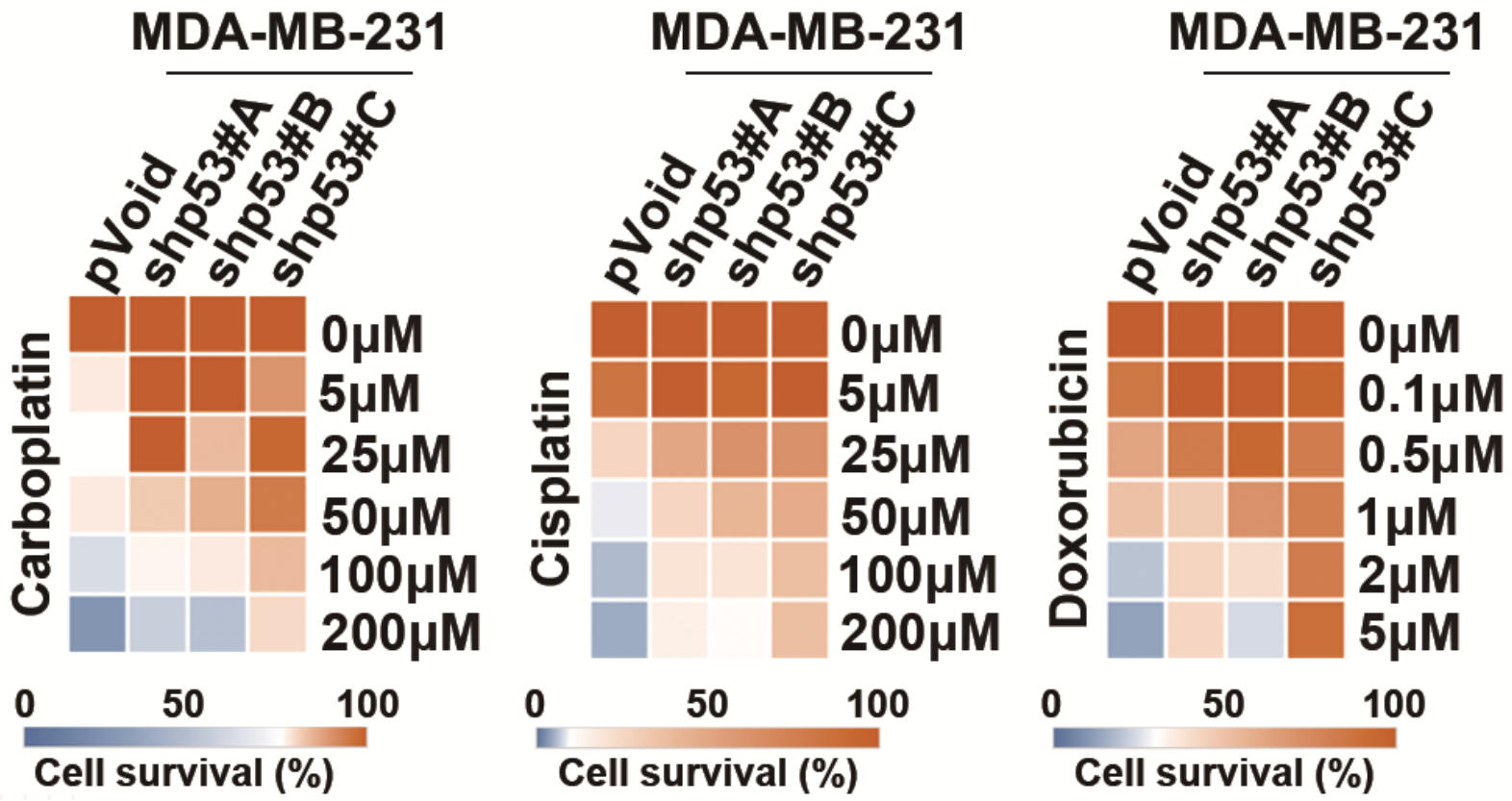
Table 4 Chemosensitivity in patients classified with P53 AmpliChip

P53 AmpliChip	Wild type ($N = 54$)	Mutant ($N = 61$)	Total ($N = 115$)
pCR	3	16	19
npCR	4	6	10
RD	47	39	86
pCR plus npCR	7/54 (13 %)	22/61 (36 %)	29/115 (25 %)

pCR pathologic complete response, *npCR* near-pathologic complete response, *RD* residual disease

Med Oncol. 2014 Oct;31(10):163.
Lancet Oncol 2011;12:527–539.

Silencing p53 reduced the chemo-sensitivity in mutp53-expressing TNBC



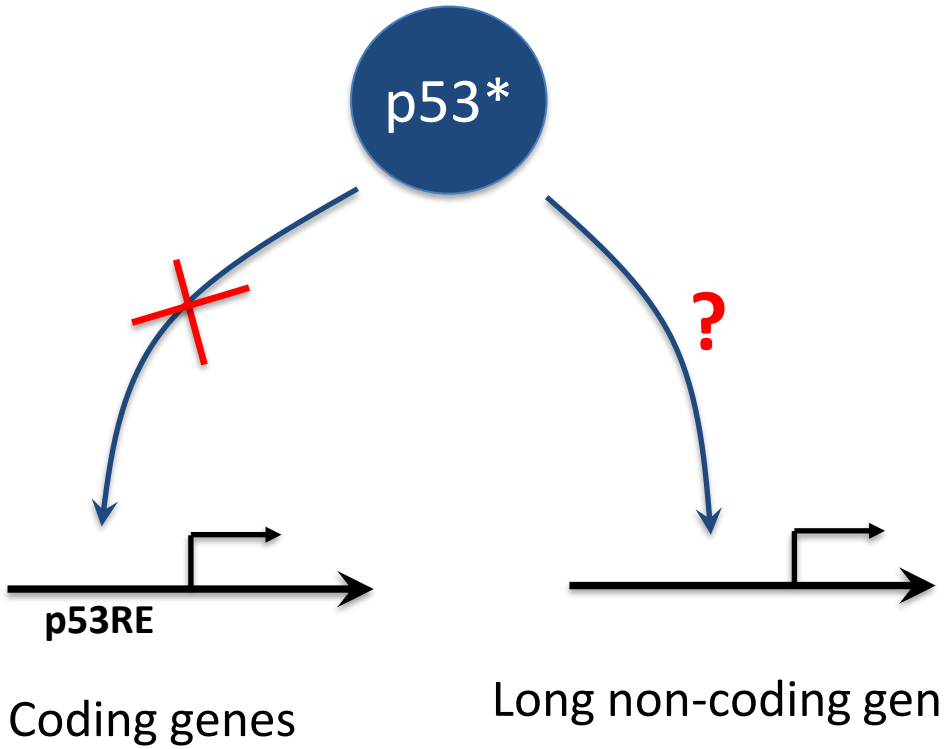
MDA-MB-231 : p53 R280K

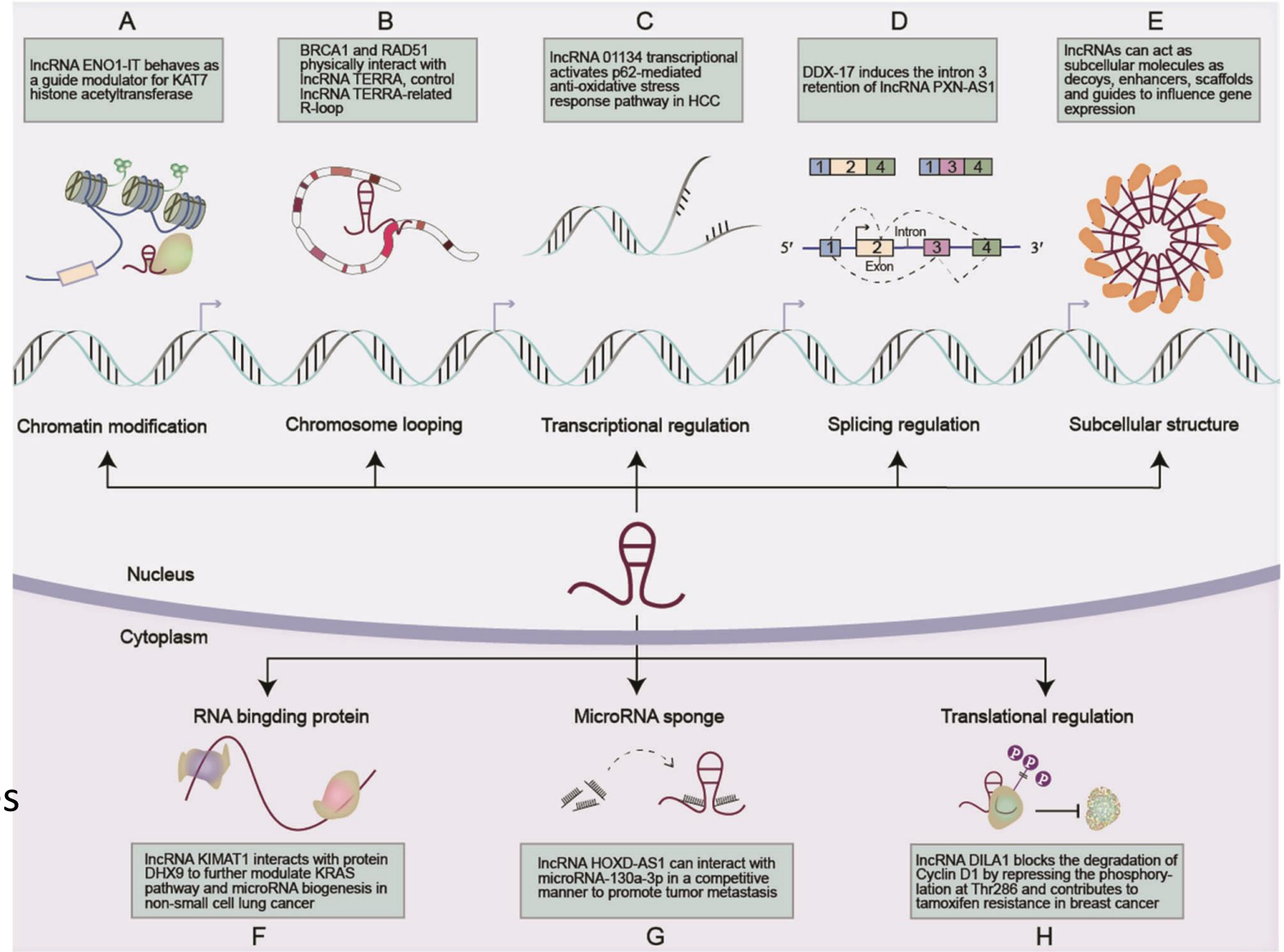
Molecular Therapy-Nucleic Acids 2021

Q1 :

Why p53 mutants still mediate chemotherapy-induced cell death?

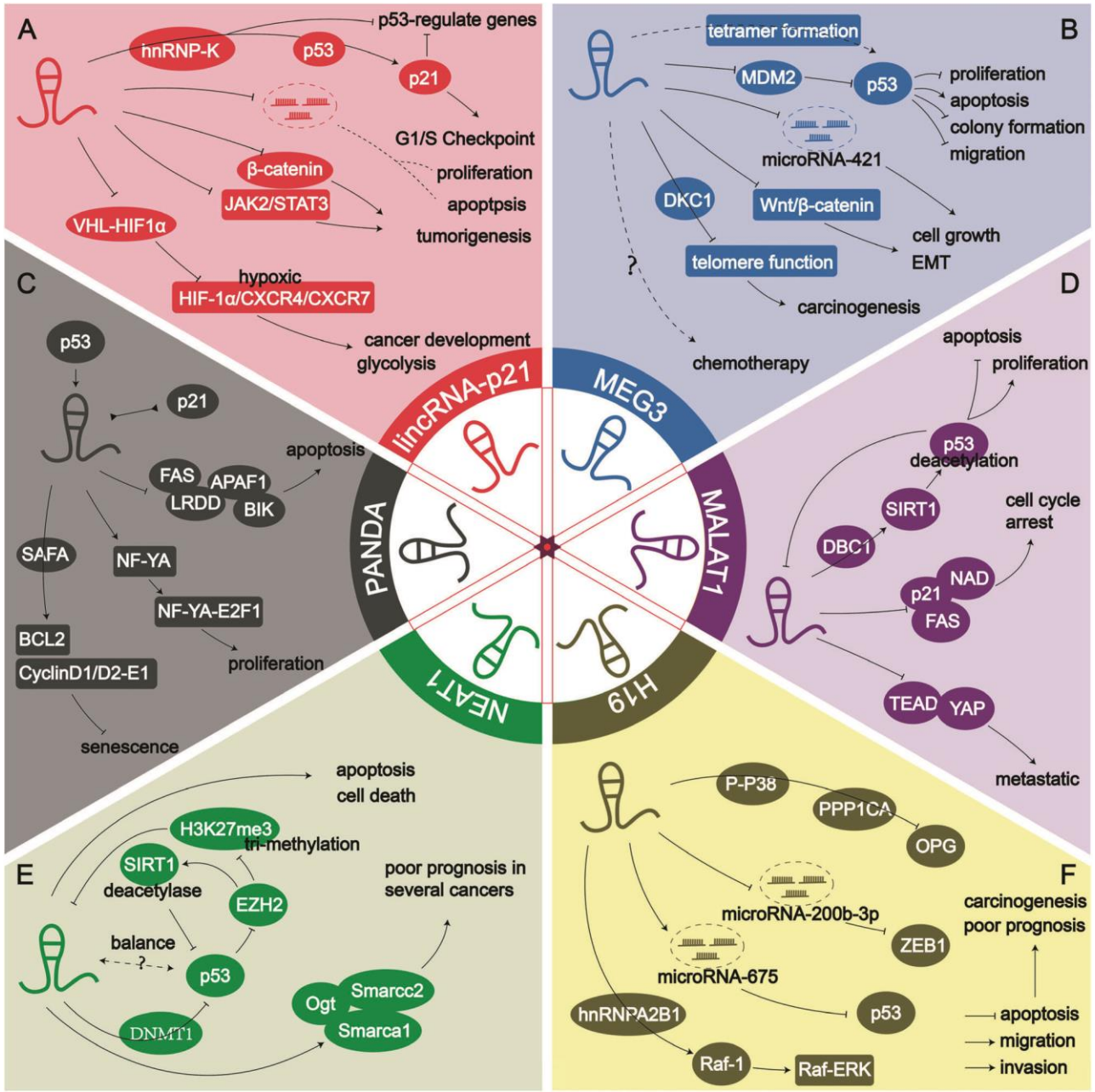
The Molecular Mechanisms of lncRNAs (Long non-coding RNA)





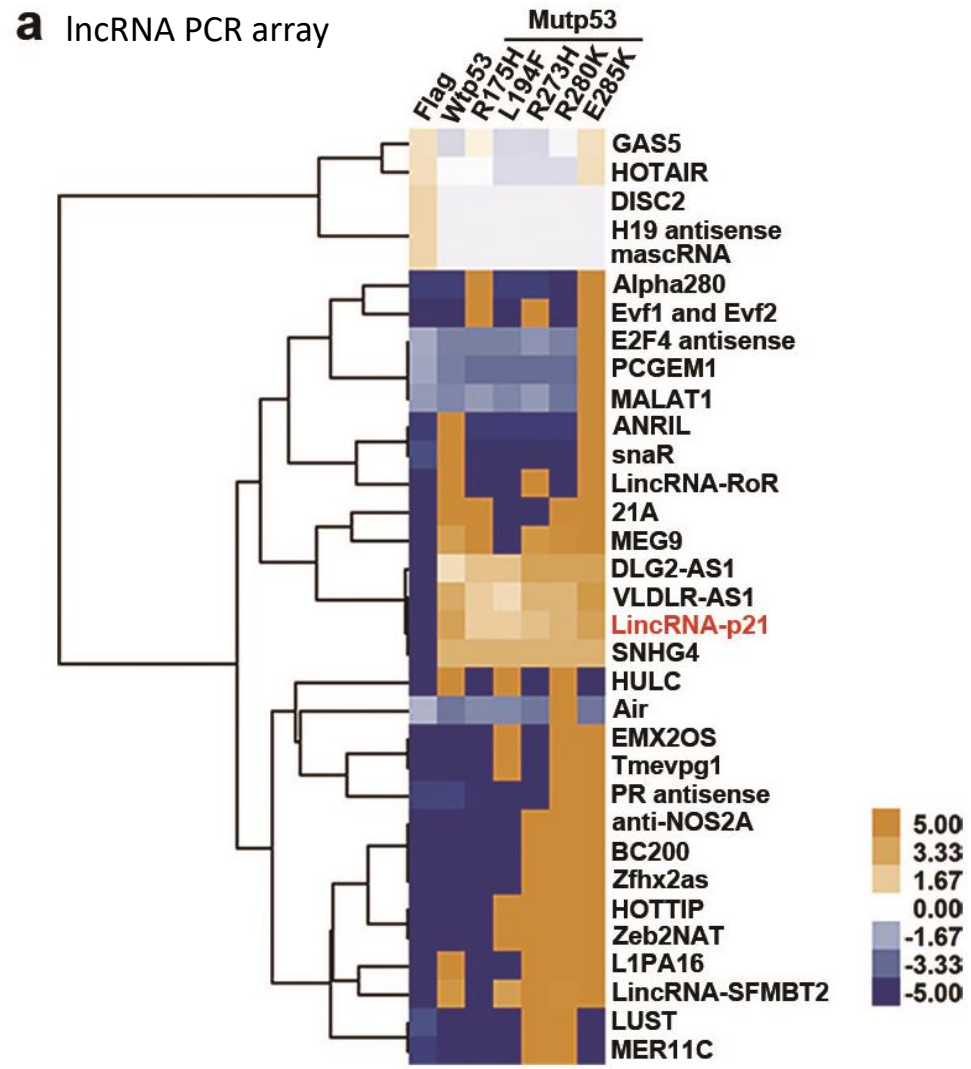
Cancer Gene Therapy (2023) 30:1456 – 1470

The p53-related lncRNA in cancer.



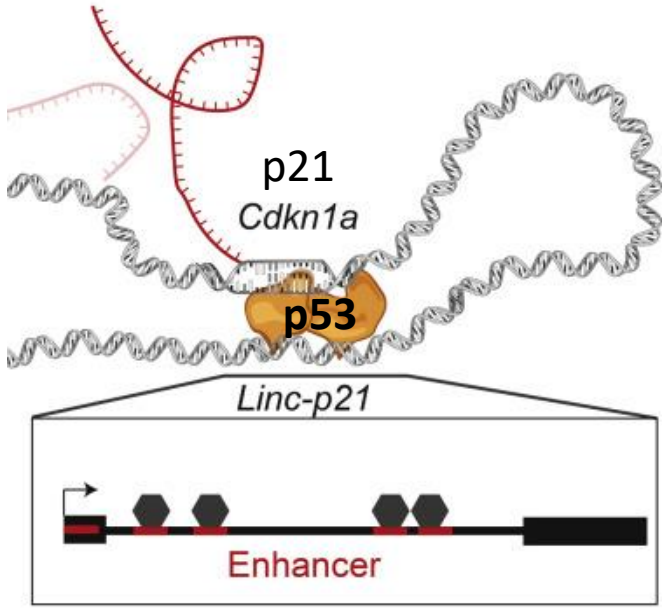
Cancer Gene Therapy (2023) 30:1456 – 1470

mutp53 mediates lincRNA-p21 expression



Molecular Therapy-Nucleic Acids 2021

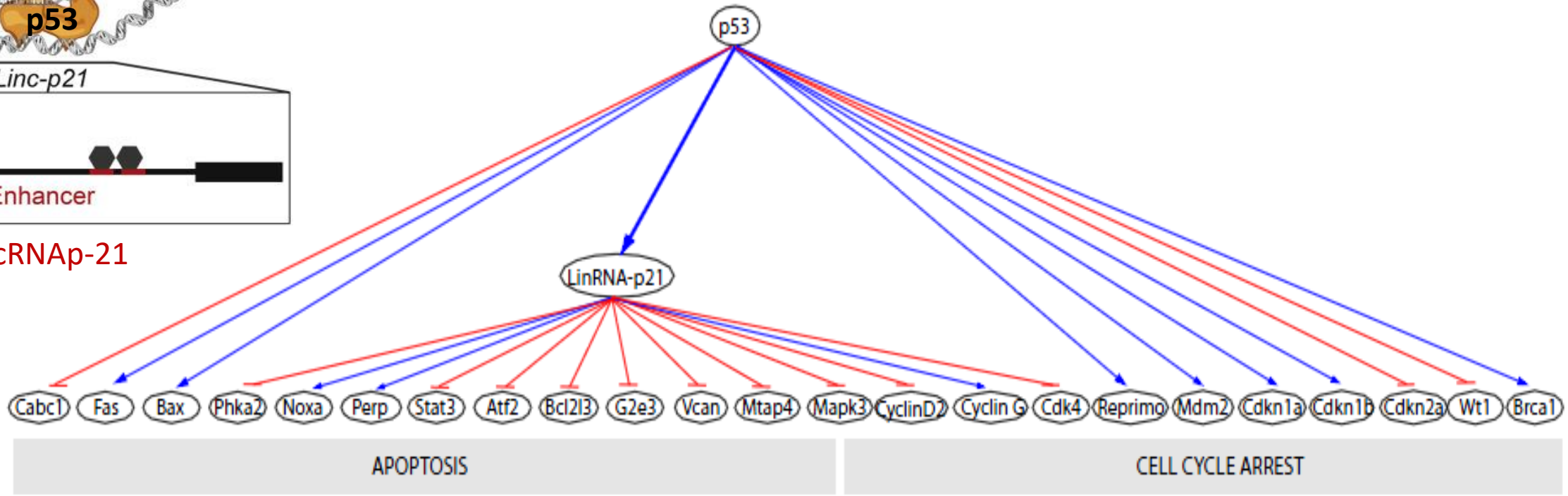
LincRNA-p21 mediates global p53-dependent gene repression



lincRNAp-21

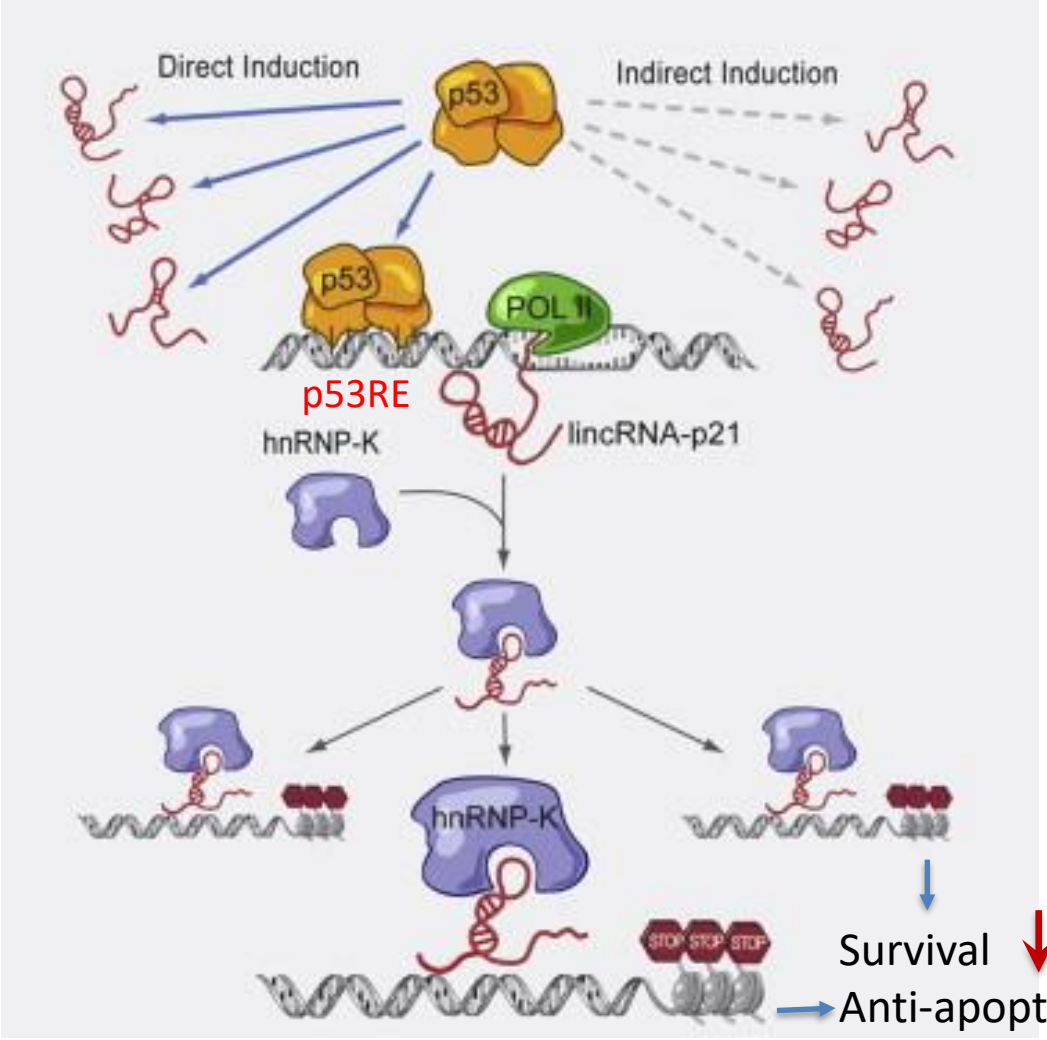
A Large Intergenic Noncoding RNA Induced by p53 Mediates Global Gene Repression in the p53 Response

Maite Huarte,[1,2,*] Mitchell Guttman,[1,3] David Feldser,[3,4] Manuel Garber,[1] Magdalena J. Koziol,[1,2] Daniela Kenzelmann-Broz,[5,6] Ahmad M. Khalil,[1,2] Or Zuk,[1] Ido Amit,[1] Michal Rabani,[1] Laura D. Attardi,[5,6] Aviv Regev,[1,3] Eric S. Lander,[1,3,7] Tyler Jacks,[3,4] and John L. Rinn[1,2,*]



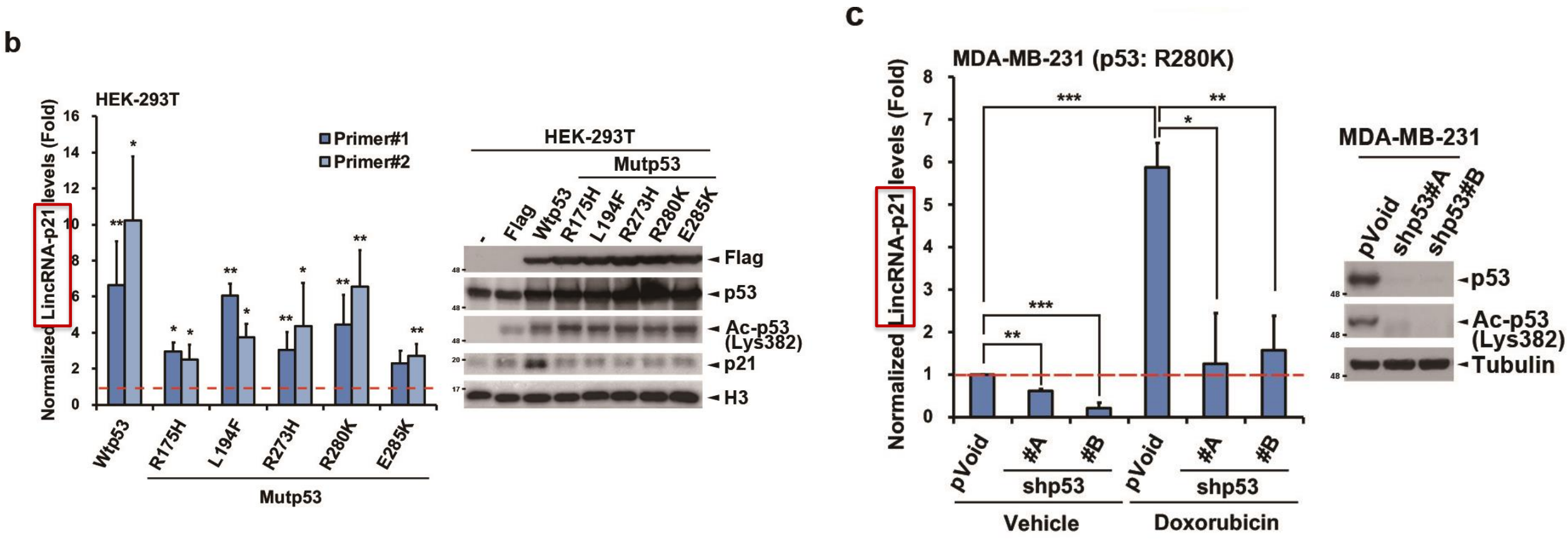
Cell. 142(3):409-19, 2010

LincRNA-p21 mediates global p53-dependent gene suppression



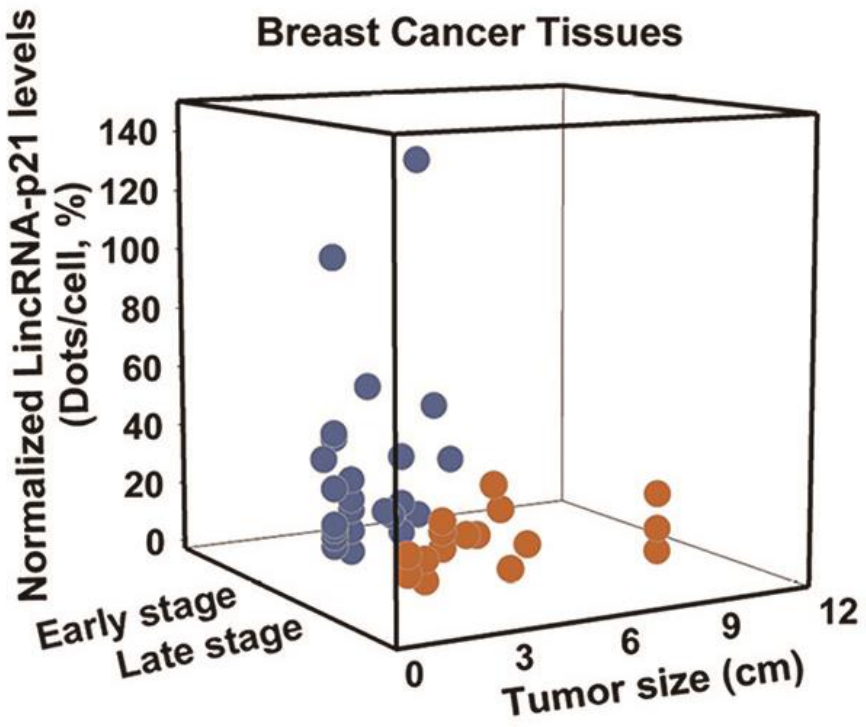
Cell. 142(3):409-19, 2010

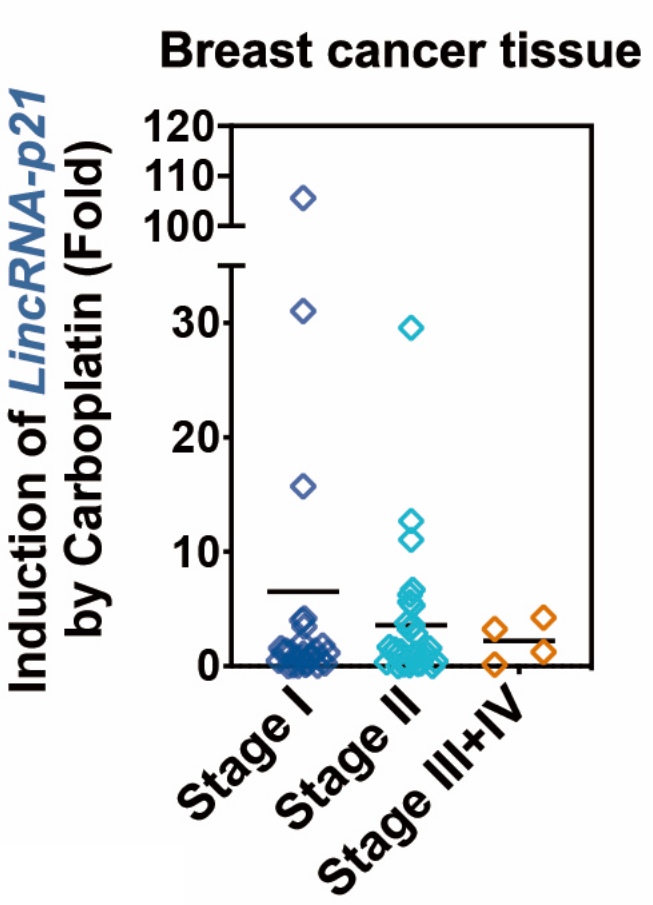
mutp53 mediates lincRNA-p21 expression

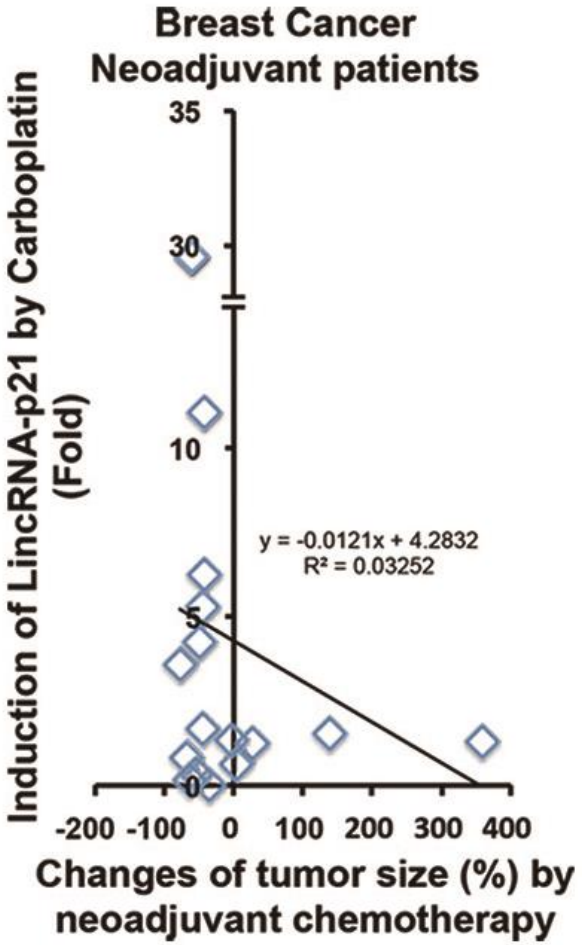


Molecular Therapy-Nucleic Acids 2021

LincRNA-p21 expression is associated with chemo-response



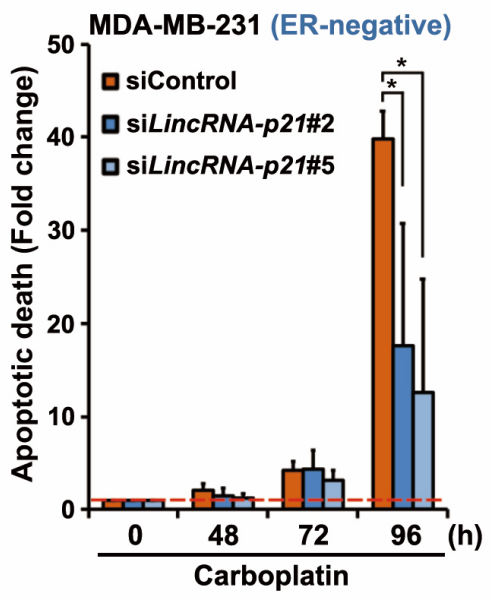


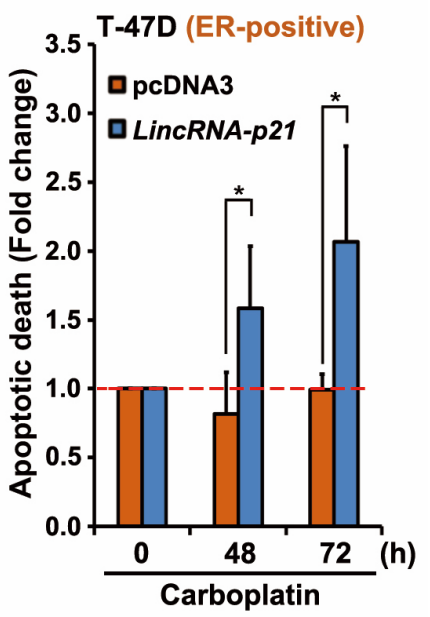


Molecular Therapy-Nucleic Acids 2021

Induction of lincRNA-p21 reduces chemoresistance of breast tumors

T47D: Mutp53-expressing breast cancer cells





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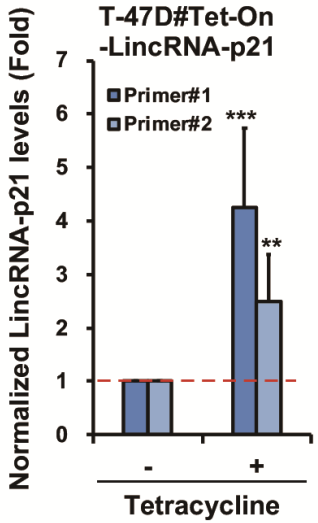

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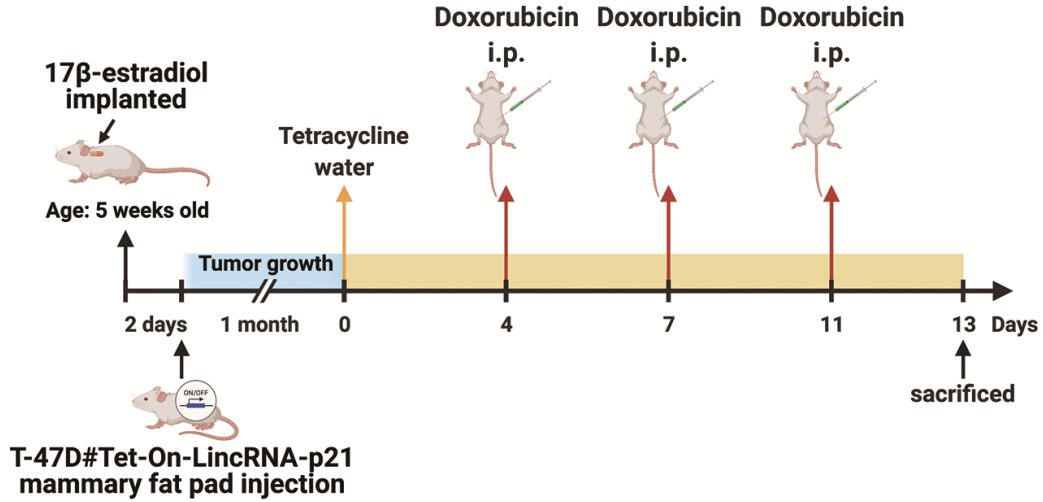

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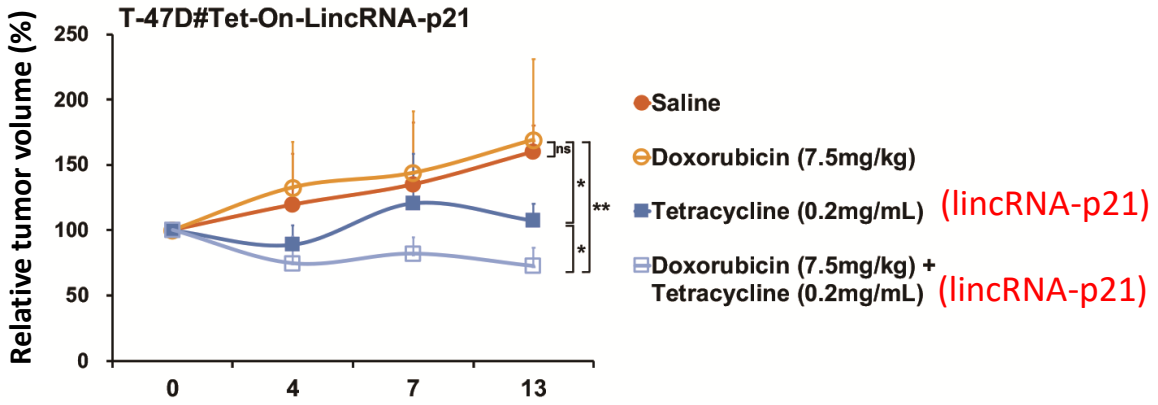


Unpublished and Molecular Therapy-Nucleic Acids 2021

LincRNA-p21 expression is low in ER-positive breast cancer.

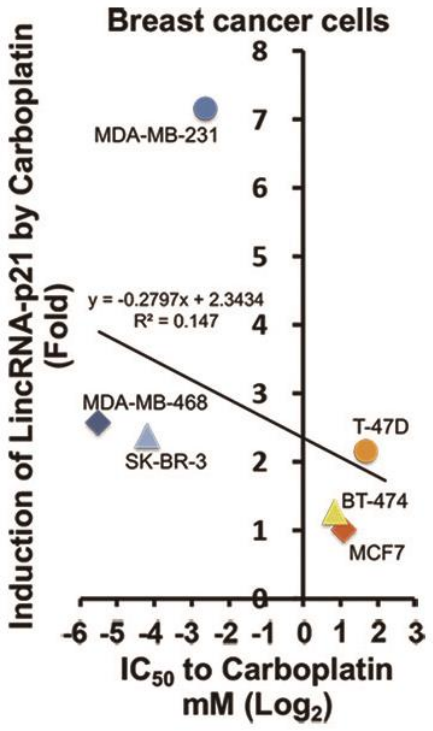


Table 2. Characteristic of Breast cancer cell lines

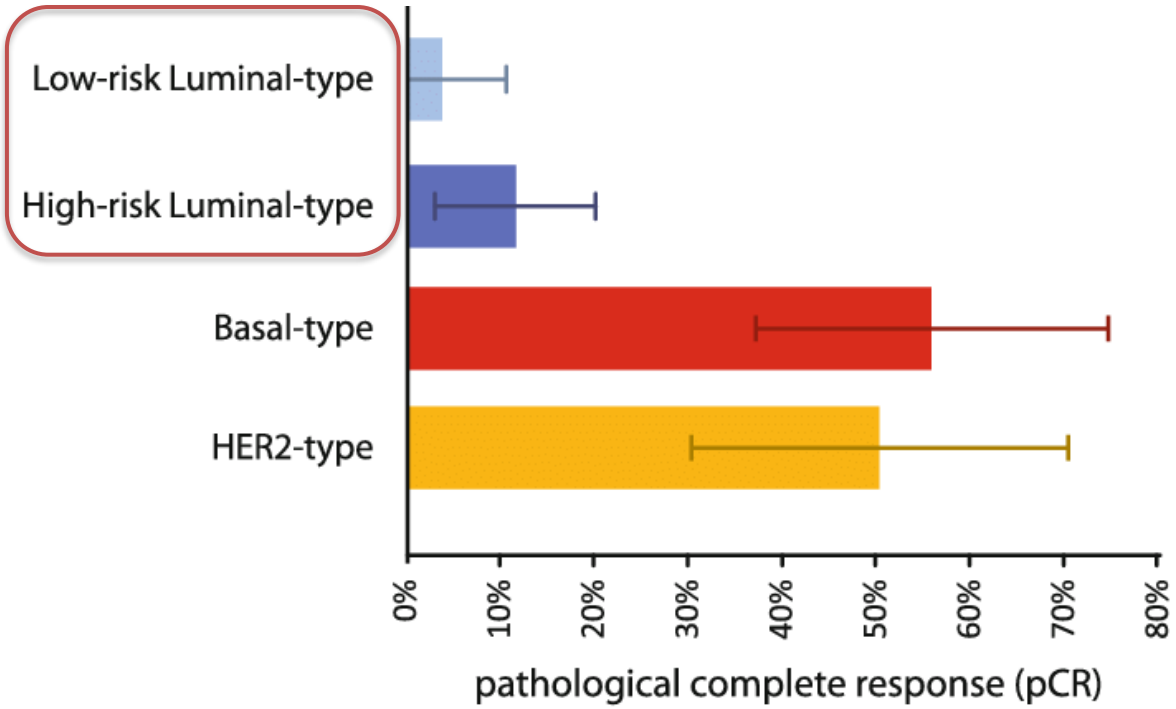
Breast Cancer Cells	ErbB2 statue	ER/PR status	Ki67 status	TP53 status	DDB2 status	Carboplatin, IC50	Cisplatin, IC50	Doxorubicin, IC50	Chemo-response	lincRNAp21 Induction
MCF-7	-	+/+	Low	Wild-type	High	2.17mM	0.46mM	1.52μM	Insensitive	Low
T-47D	-	+/+	Low	Mutation (L194F)	High	3.24mM	7.58mM	4.31μM	Insensitive	Low
BT-474	+	+/+	High	Mutation (E285K)	High	1.84mM	0.63mM	15.20μM	Insensitive	Low
SK-BR-3	+	-/-	High	Mutation (R175H)	Low	55.54μM	11.15μM	0.12μM	Sensitive	High
MDA-MB-231	-	-/-	Low	Mutation (R280K)	Low	0.16mM	41.41μM	1.16μM	Sensitive	High
MDA-MB-468	-	-/-	High	Mutation (R273H)	Low	22.12μM	1.51μM	0.39μM	Sensitive	High

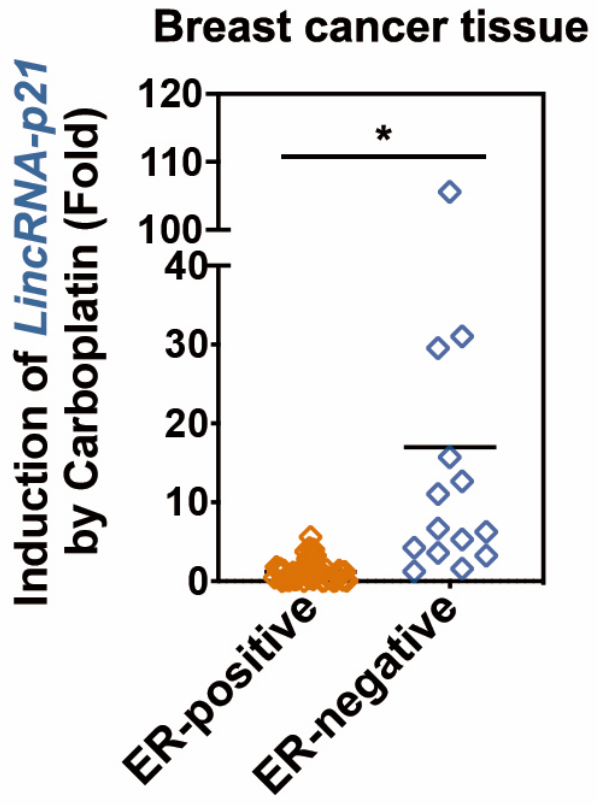
MCF-7 cells express p53wt

Molecular Therapy-Nucleic Acids 2021

Estrogen receptor is associated with chemoresistance

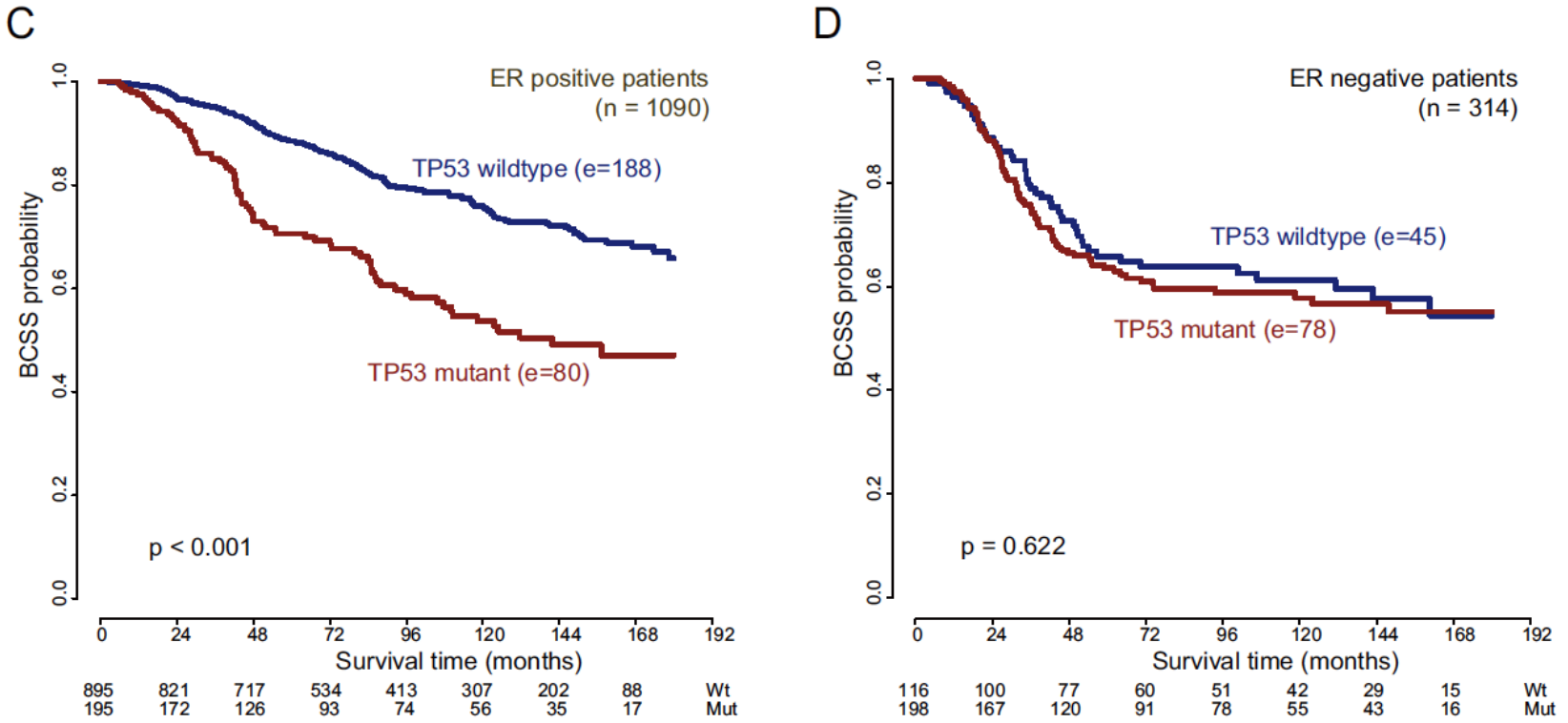
ERα-positive





Breast Cancer Res Treat (2012) 133:37–47

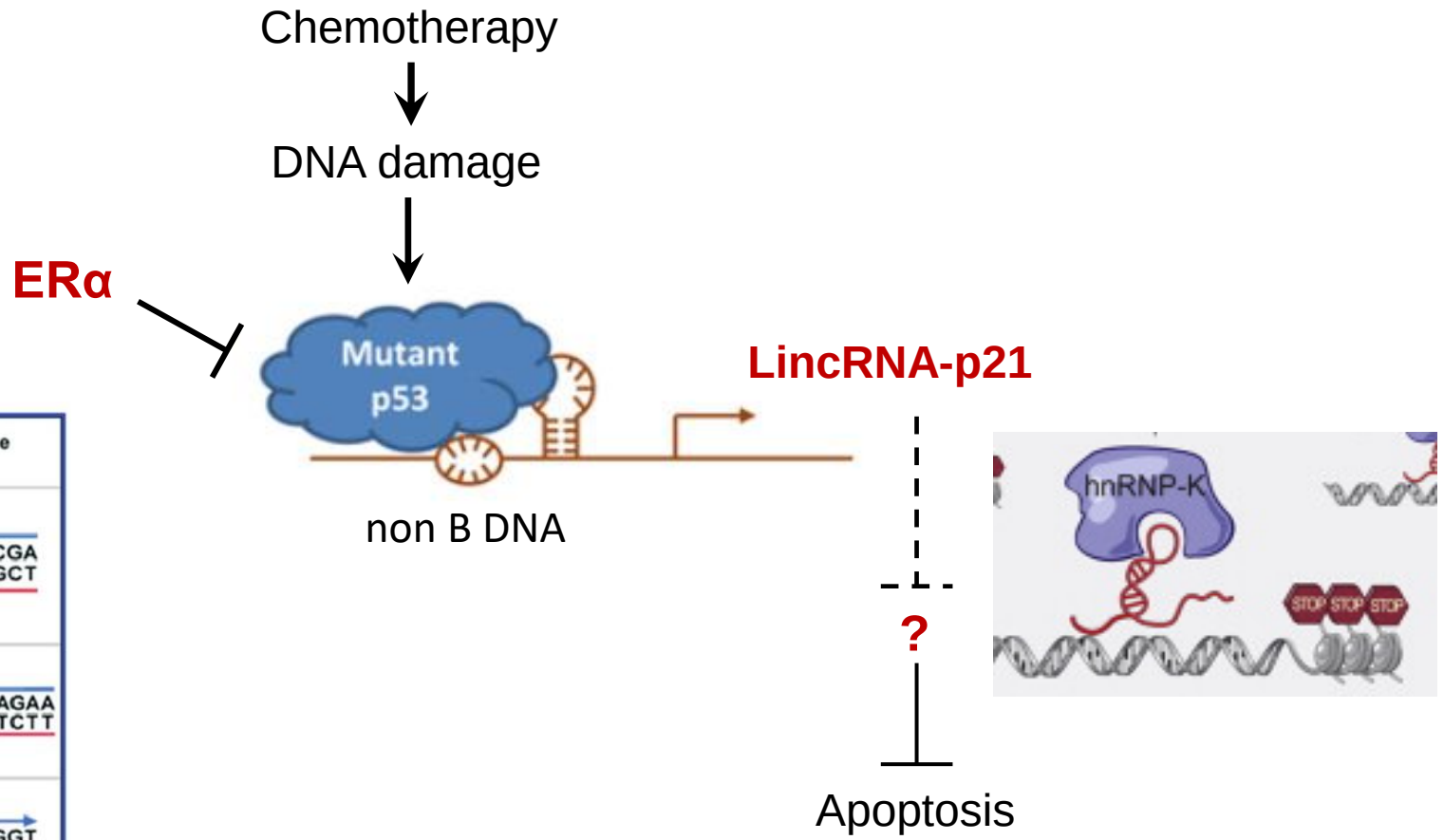
TP53 mutation predicts the poor survival rate in ER-positive but not -negative breast cancer patients



ERα ⊣ p53-lincRNA-p21 signaling axis

Clin Cancer Res. 20(13):3569-80, 2014

Summary #1



Non-B DNA

Name	Conformation	General Seq. Requirements	Sequence
Cruciform		Inverted Repeats	TCGGTACCGA AGCCATGGCT
Triplex		$(R \cdot Y)_n$ Mirror Repeats	AAGAGGGGAGAA TTCTCCCCTCTT
Slipped (Hairpin) Structure		Direct Repeats	TCGGTTCGGT AGCCAAGCCA
Tetraplex		Oligo $(G)_n$ Tracts	$AG_3(T_2AG_3)_3$ single strand
Left-handed Z - DNA	B - Z Junctions	$(YR \cdot YR)_n$	CGCGTGCGTGTG GCGCACGCACAC

G-Quadruplex

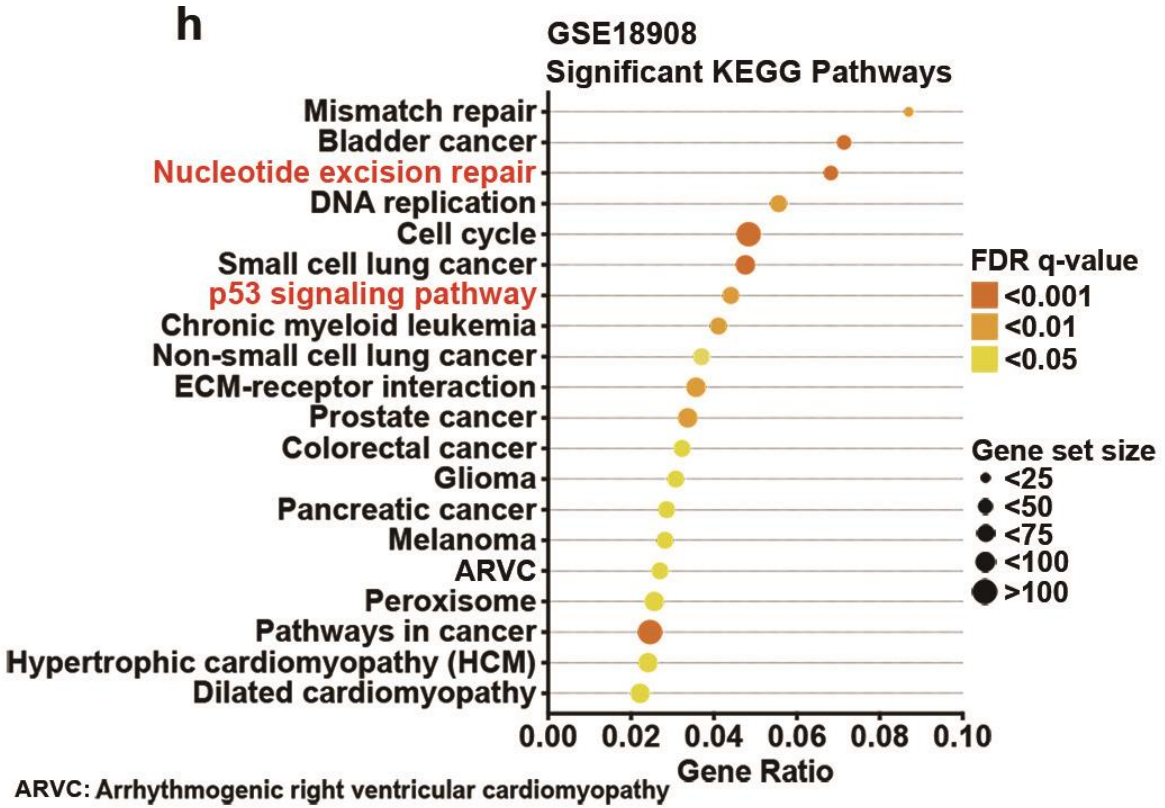
Molecular Therapy-Nucleic Acids 2021
Journal of Hazardous Materials 2024

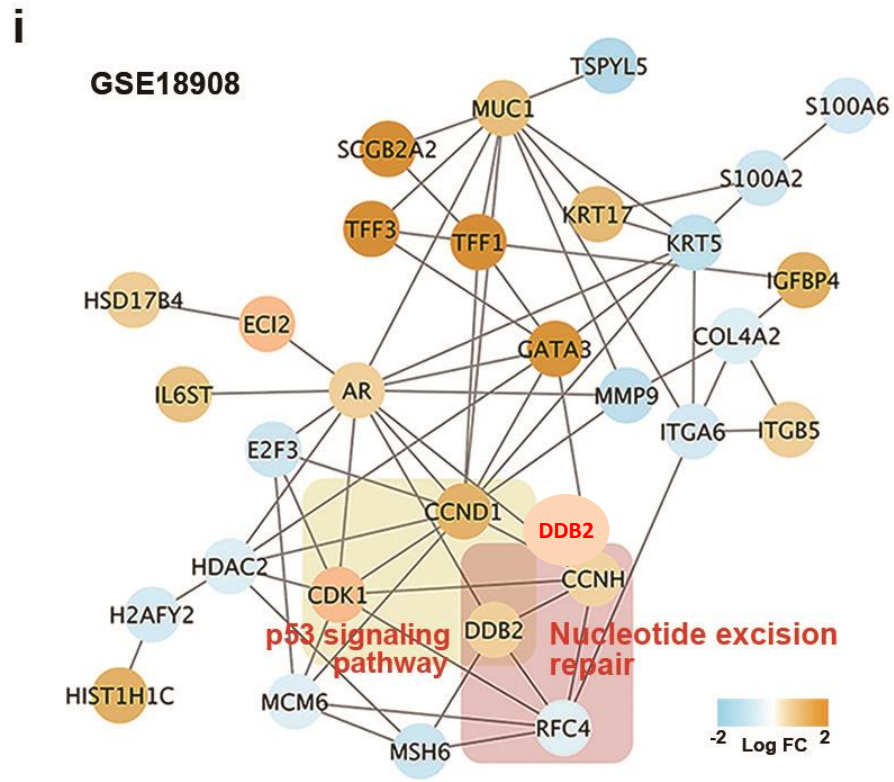
Q2 :

The downstream regulators of ERα/p53/lincRNA-p21 axis in mediating chemoresistance ?

DDB2 is a potential target gene regulated by both estrogen receptor and p53 signaling pathways

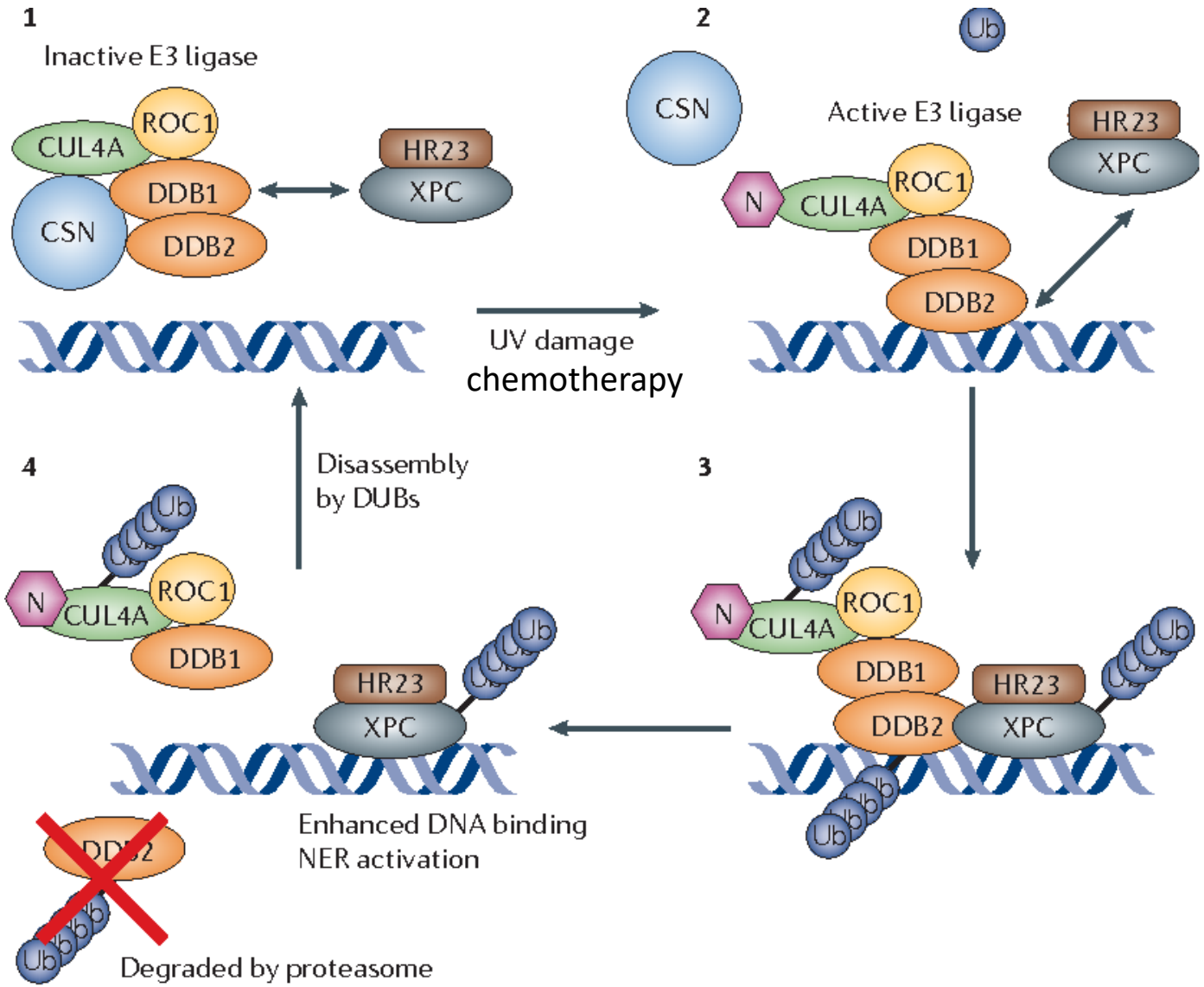
ER+/ER- breast tumor tissues





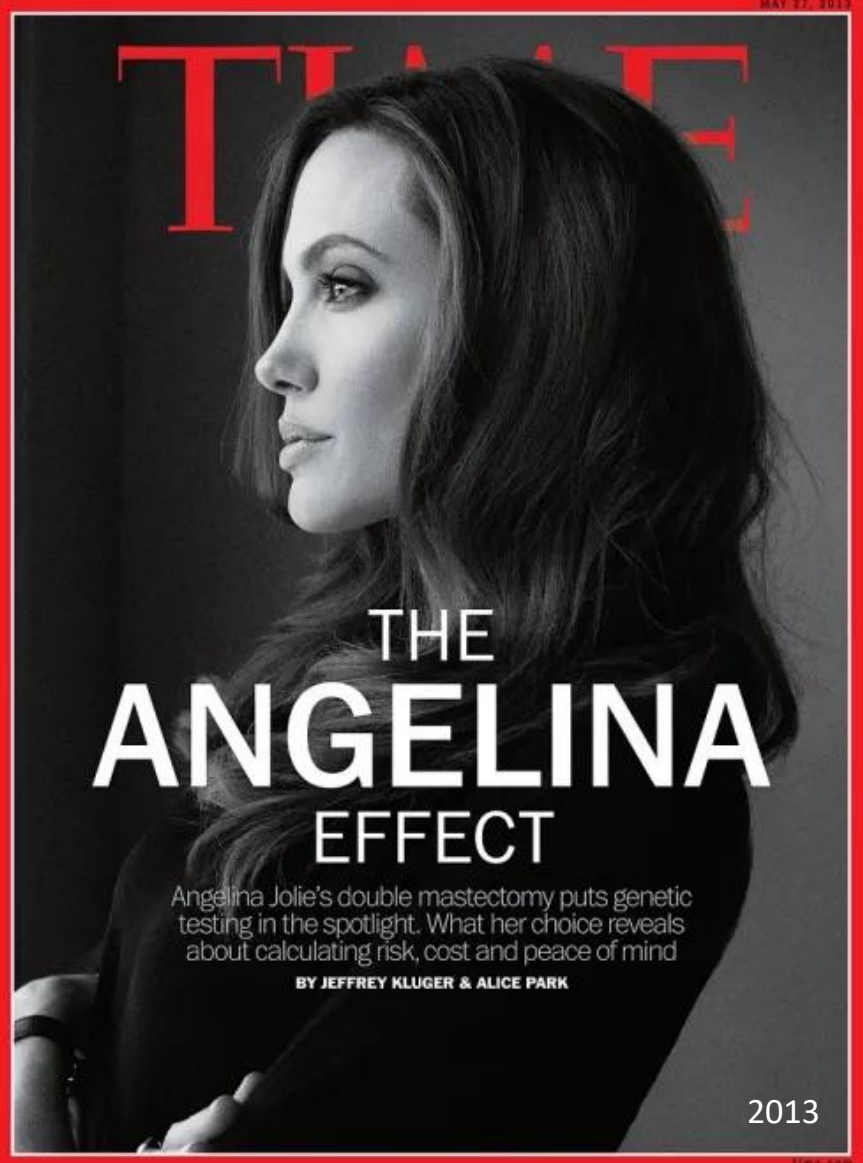
STRING:
functional protein association networks

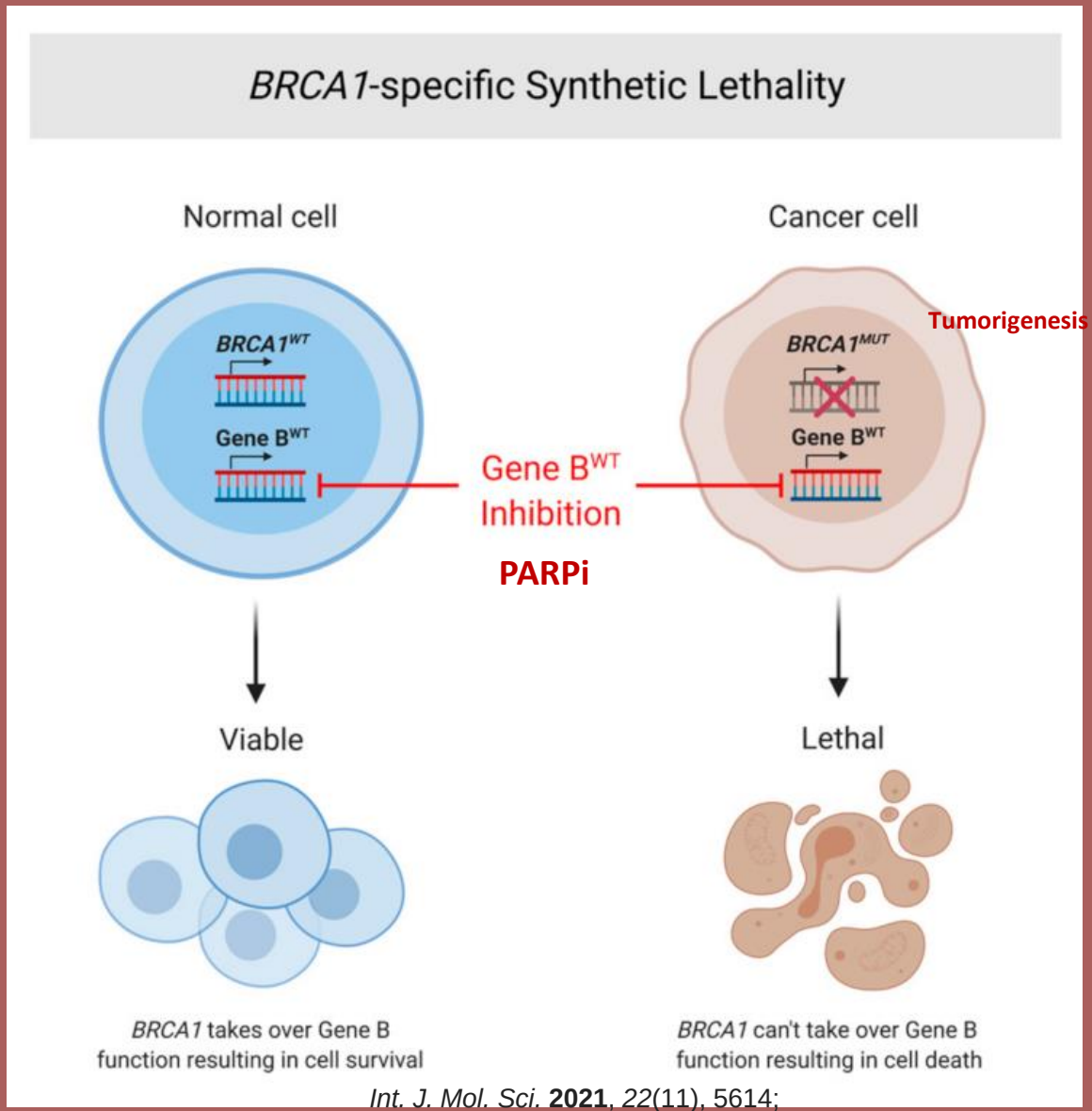
Damaged DNA-binding protein-2 (DDB2) in DNA repair



Nature Reviews Molecular Cell Biology 2006

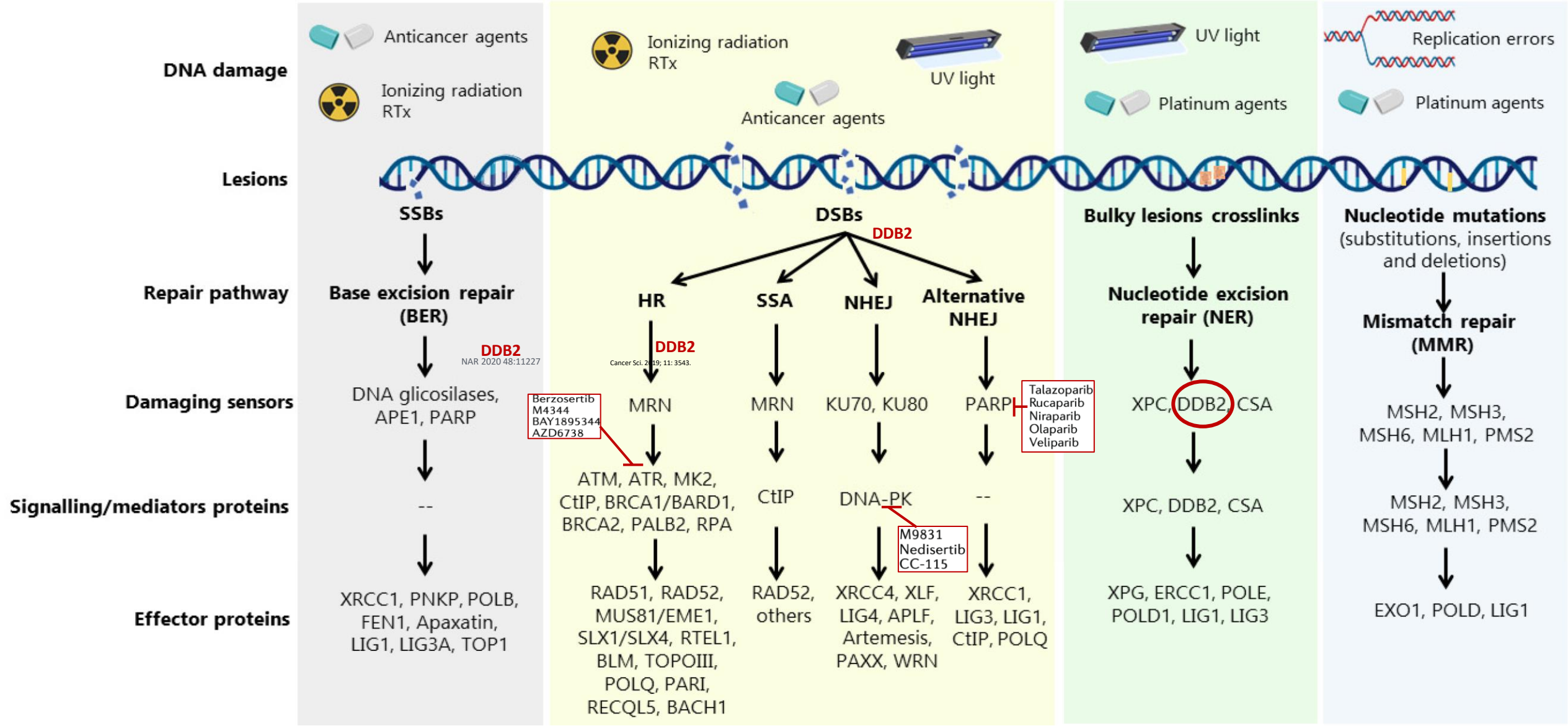
Initiation of DNA Damage response-targeting era





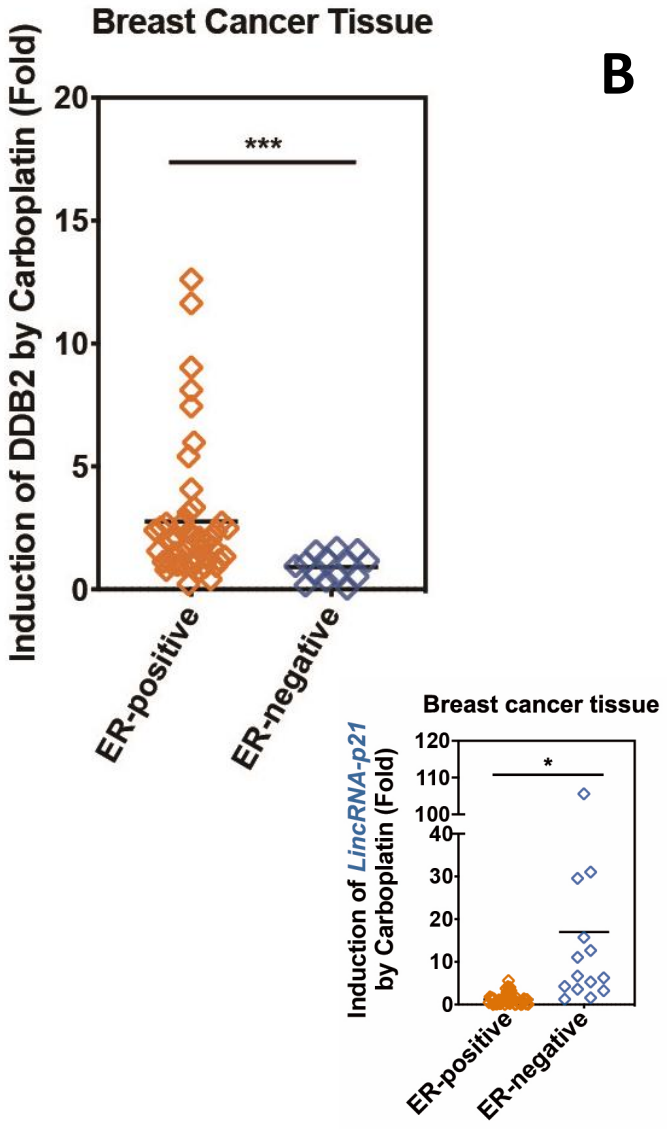
Int. J. Mol. Sci. **2021**, *22*(11), 5614;

DNA damage response (DDR) pathways being targeted in the clinic.



DDB2 positively correlates with ERα expression

A



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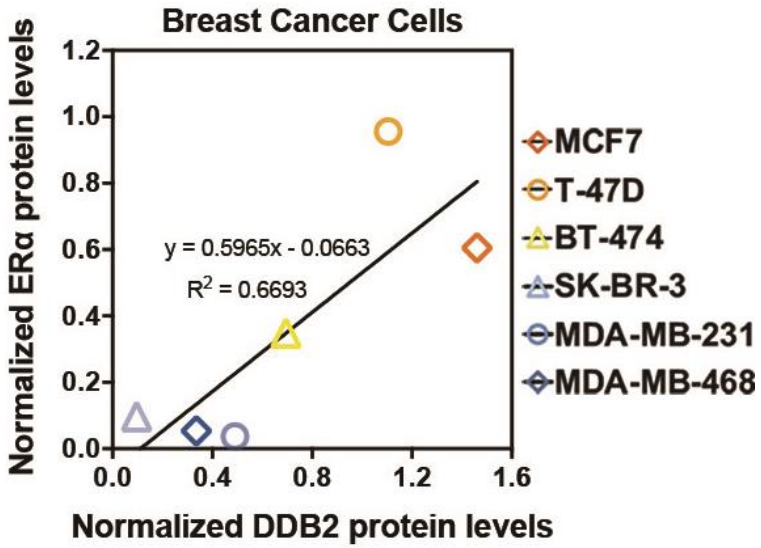

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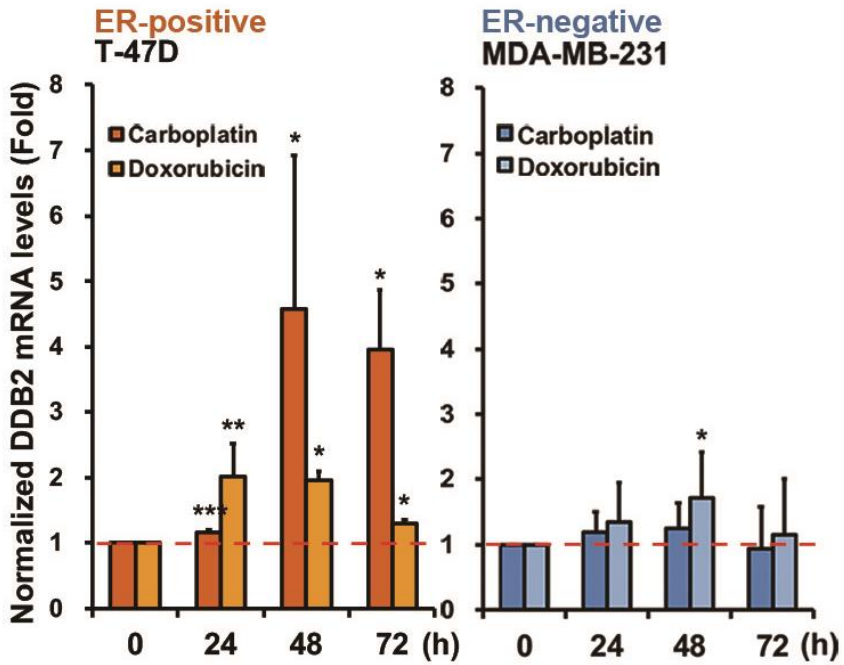


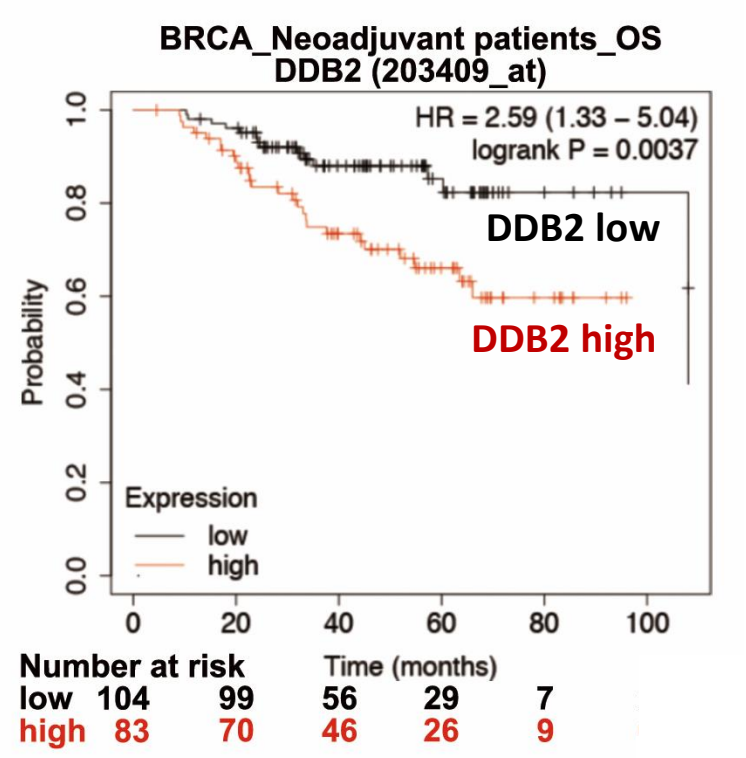
DDB2 high expression predicts chemotherapy resistance

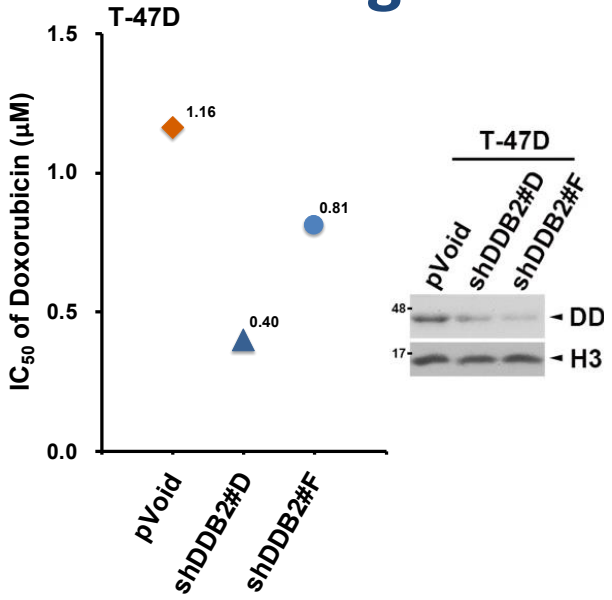
Marker expression	Pathological responders (%) *N* = 27[a]	Pathological nonresponders (%) *N* = 13[a]	*P* value	Accuracy (%)
DDB2				
Positive	4 (14.8)	8 (61.5)	0.0065	77.5
Negative	23 (84)	5 (33)		
ERCC1				
Positive	5 (18.5)	8 (61.5)	0.029	75.0
Negative	22 (81.5)	5 (42)		
DDB2 and/or ERCC1-positive	7 (25.9)	13 (100)	<0.0001	82.5
DDB2 and ERCC1-negative	20 (72)	0 (0)		

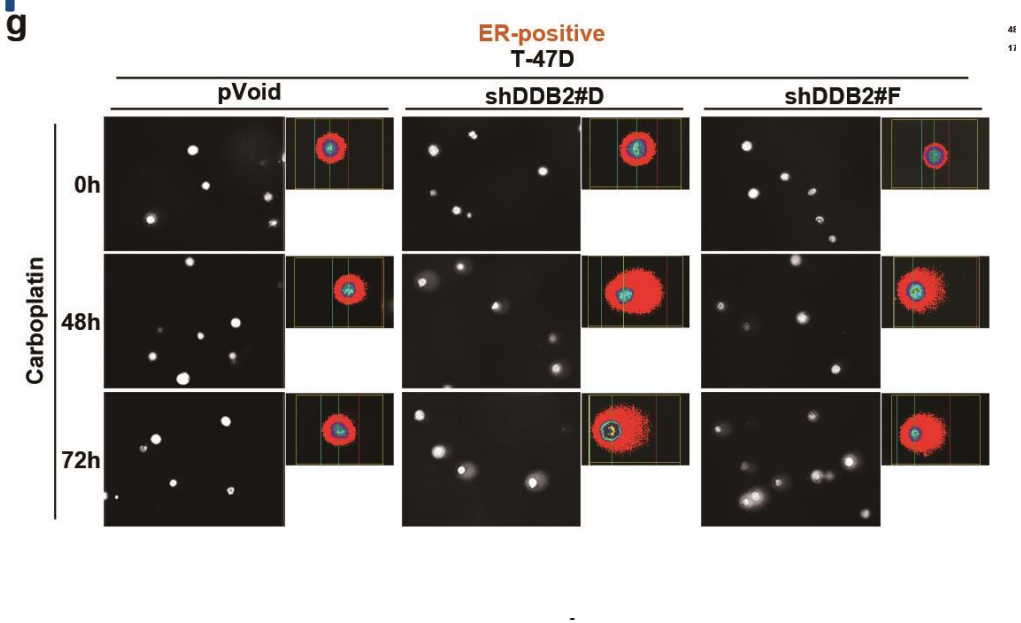
[a]Pretreatment biopsy specimens were available for analysis in 40 out of 43 patients with neoadjuvant chemotherapy

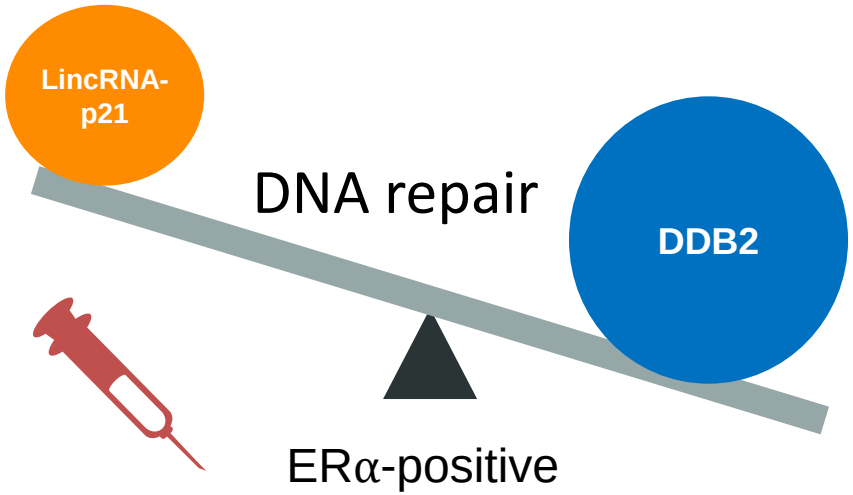
Cancer Chemother Pharmacol **71**, 789–797 (2013).

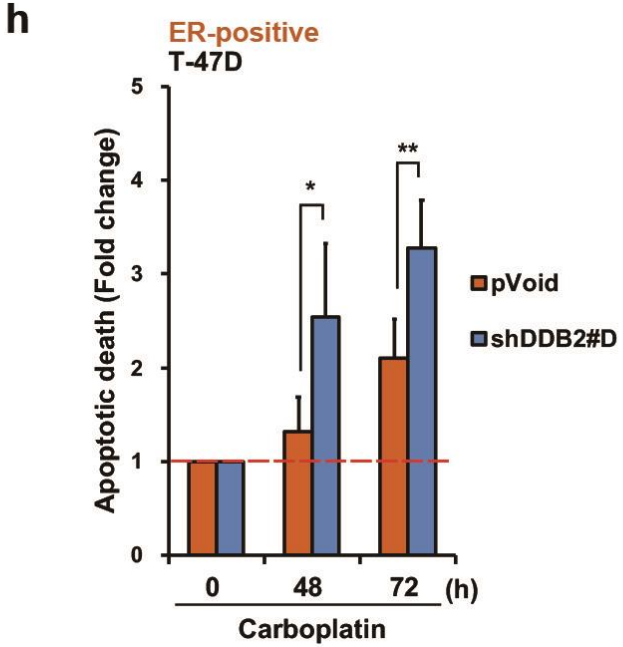
ERα-associated DDB2 expression contributes to chemoresistance by increasing DNA repair

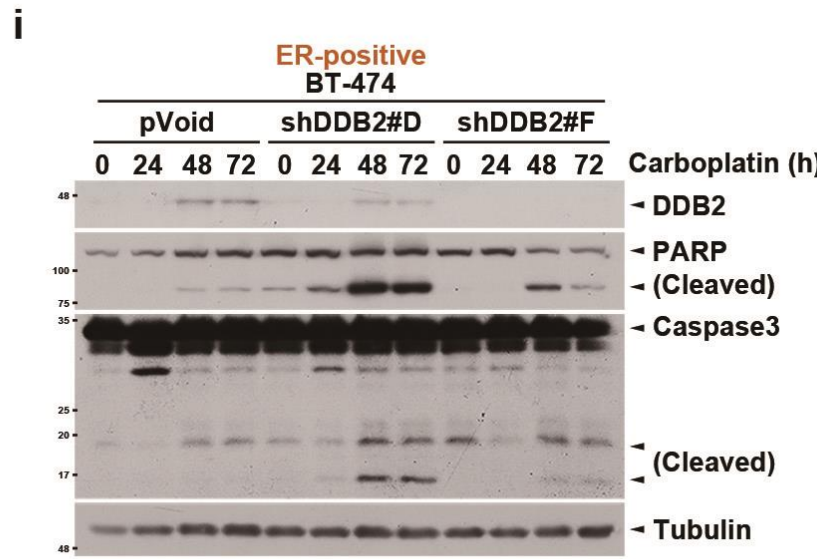




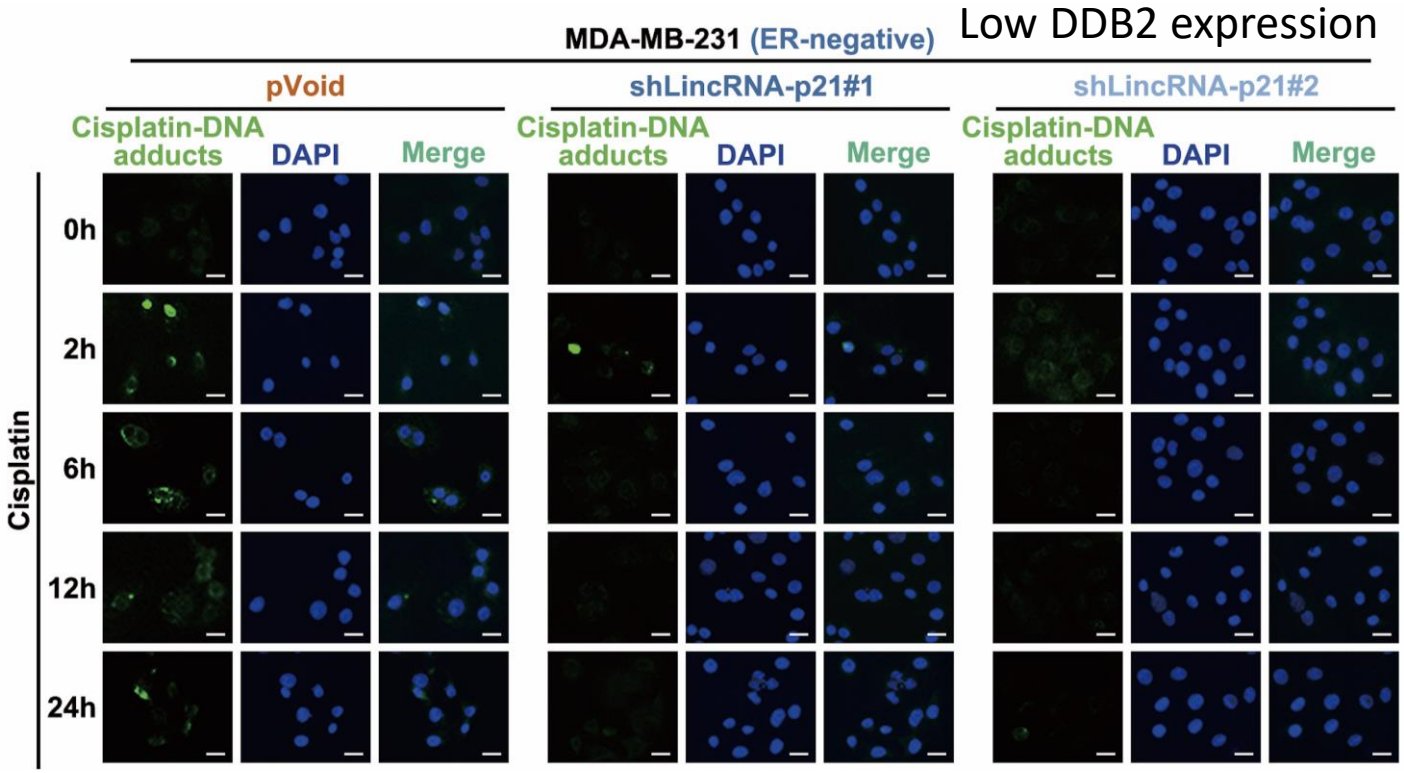


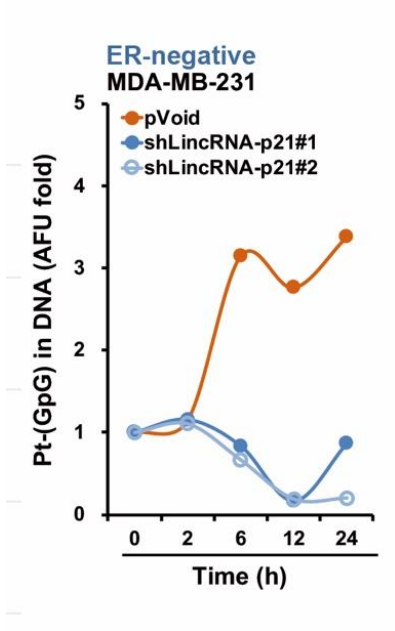






Silence of lincRNA-p21 prevents cisplatin-induced DNA damage



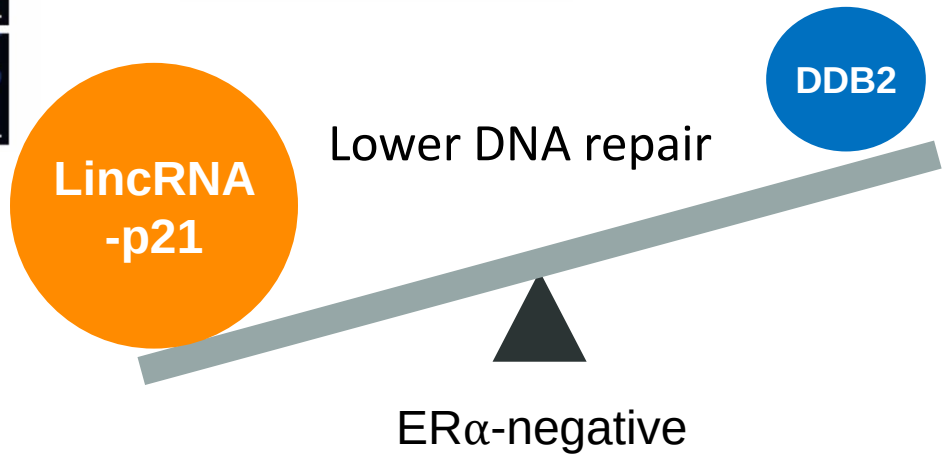


LincRNA-p21

?

DDB2

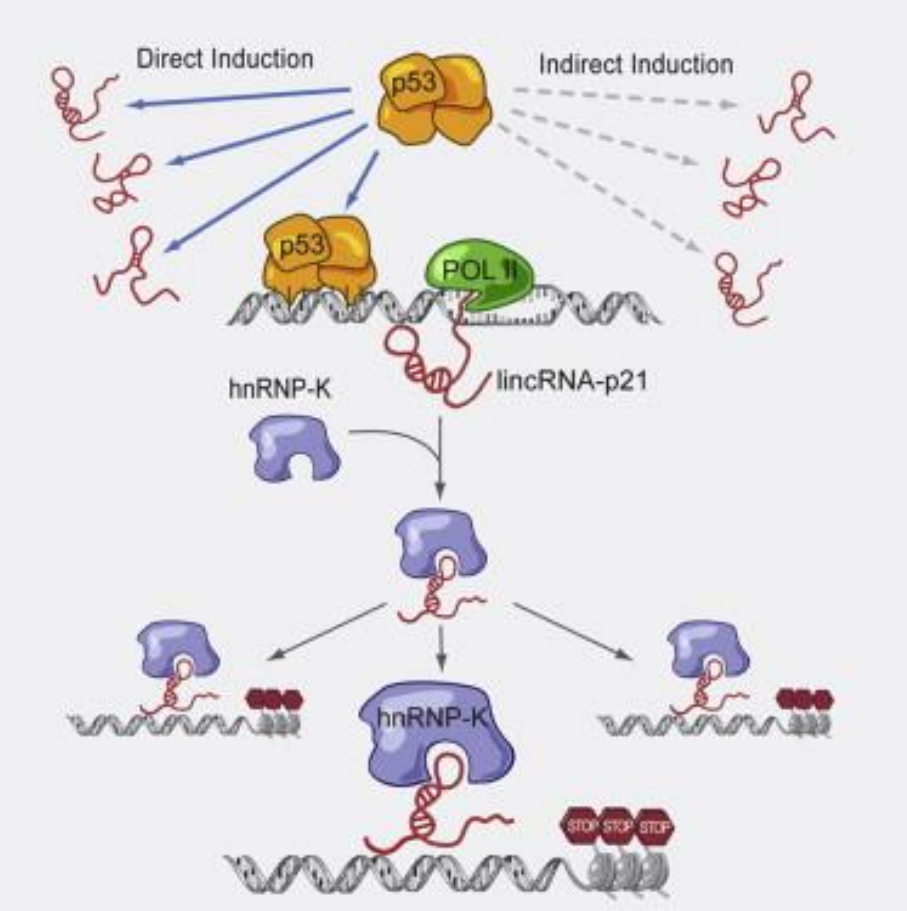
Damaged DNA repair

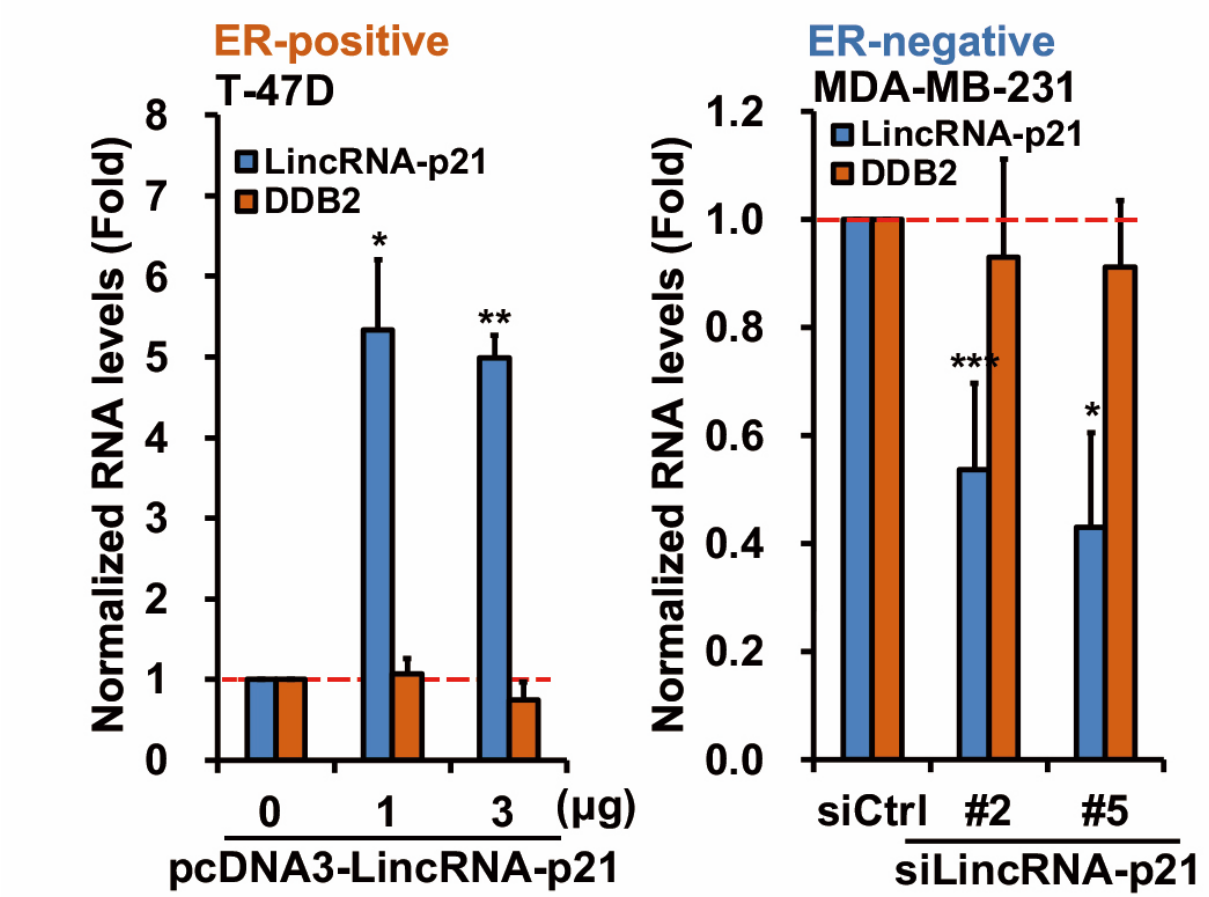


Q3 :

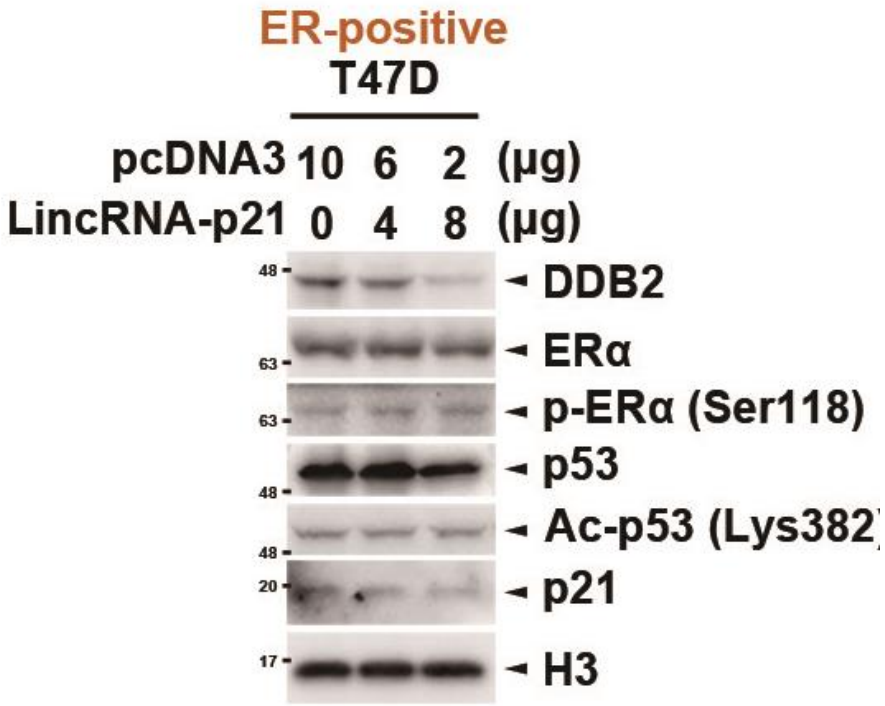
The gene regulation of DDB2 by lincRNA-p21?

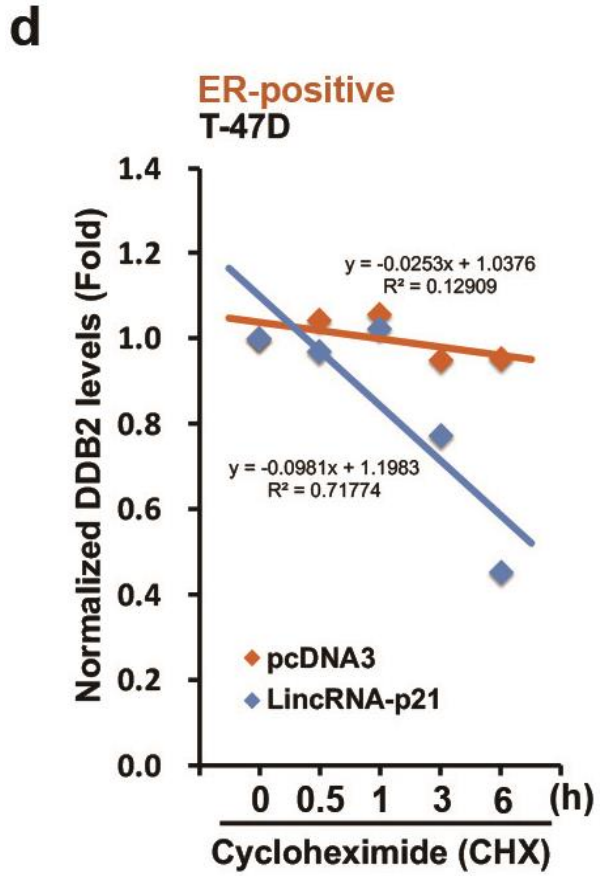
LincRNA-p21 does not suppress the mRNA level of DDB2





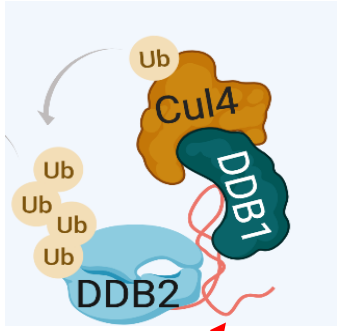
LincRNA-p21 reduces DDB2 protein stability

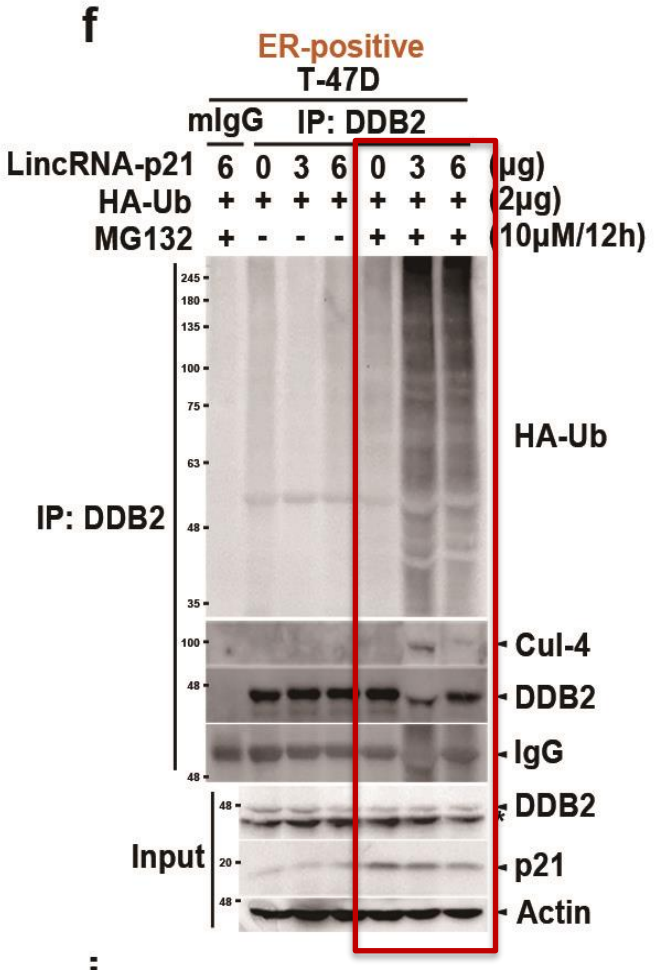


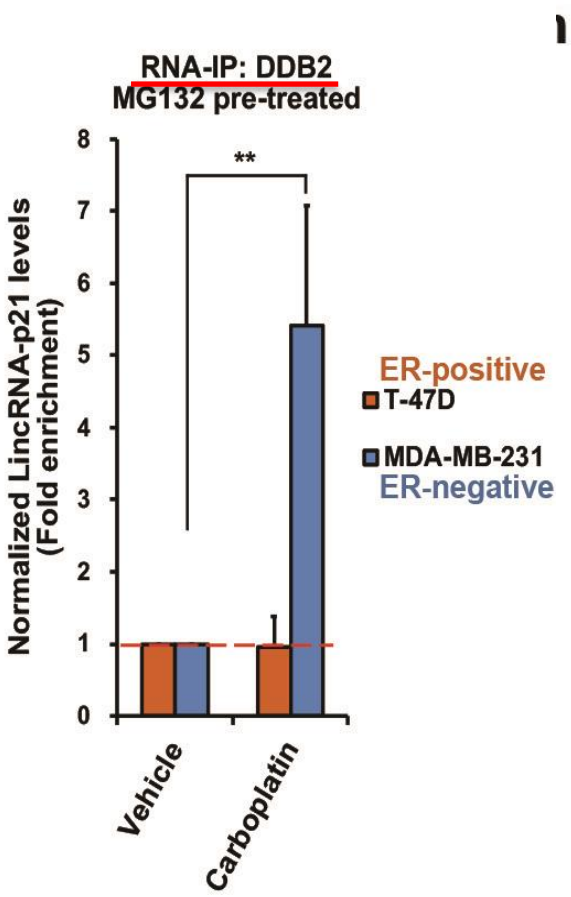


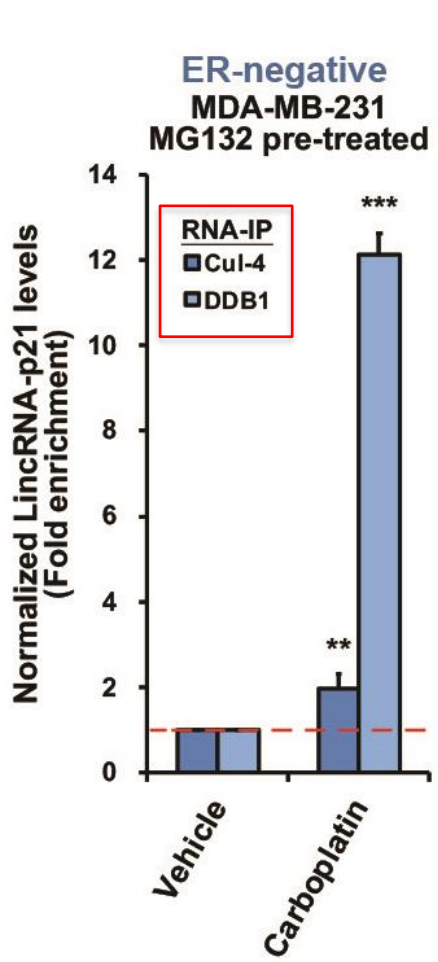
He et et al., Manuscript in preparation

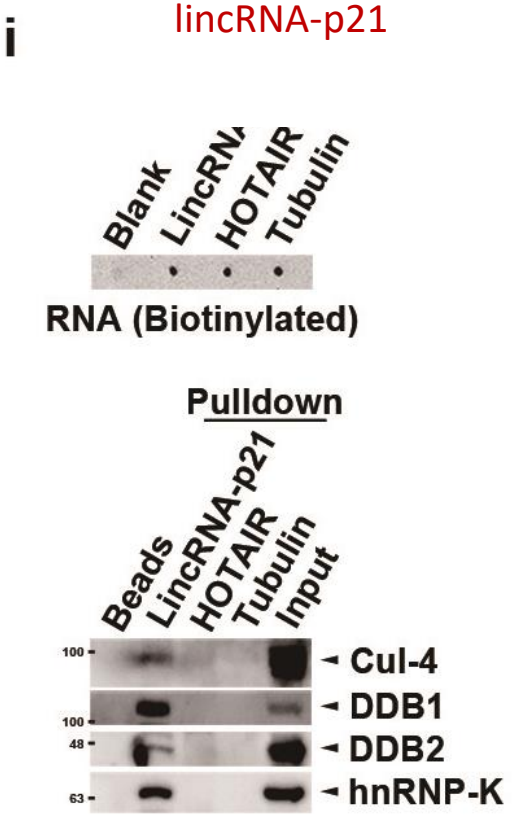
LincRNA-p21 enhances DDB2 protein ubiquitination and degradation by acting as a scaffold of CUL-4/DDB1/DDB2 complex





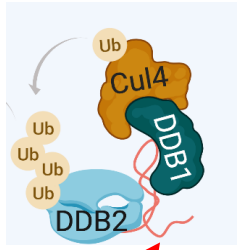




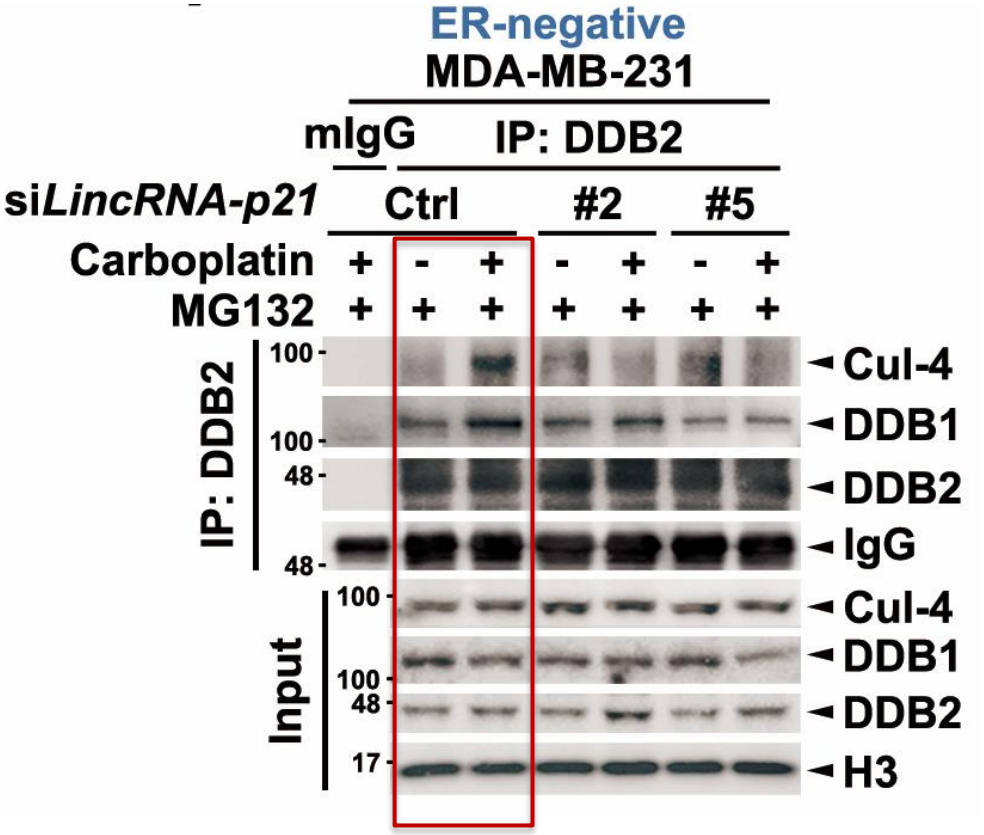


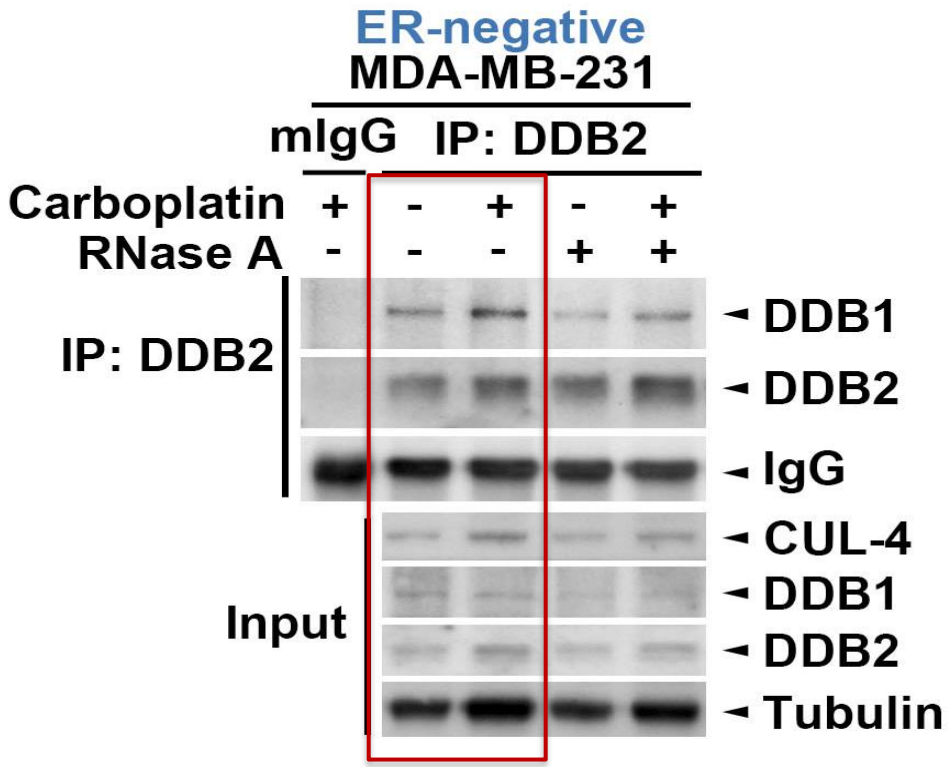
He et et al., Manuscript in preparation

LincRNA-p21 enhances DDB2 protein ubiquitination and degradation by acting as a scaffold of CUL-4/DDB1/DDB2 complex



lincRNA-p21

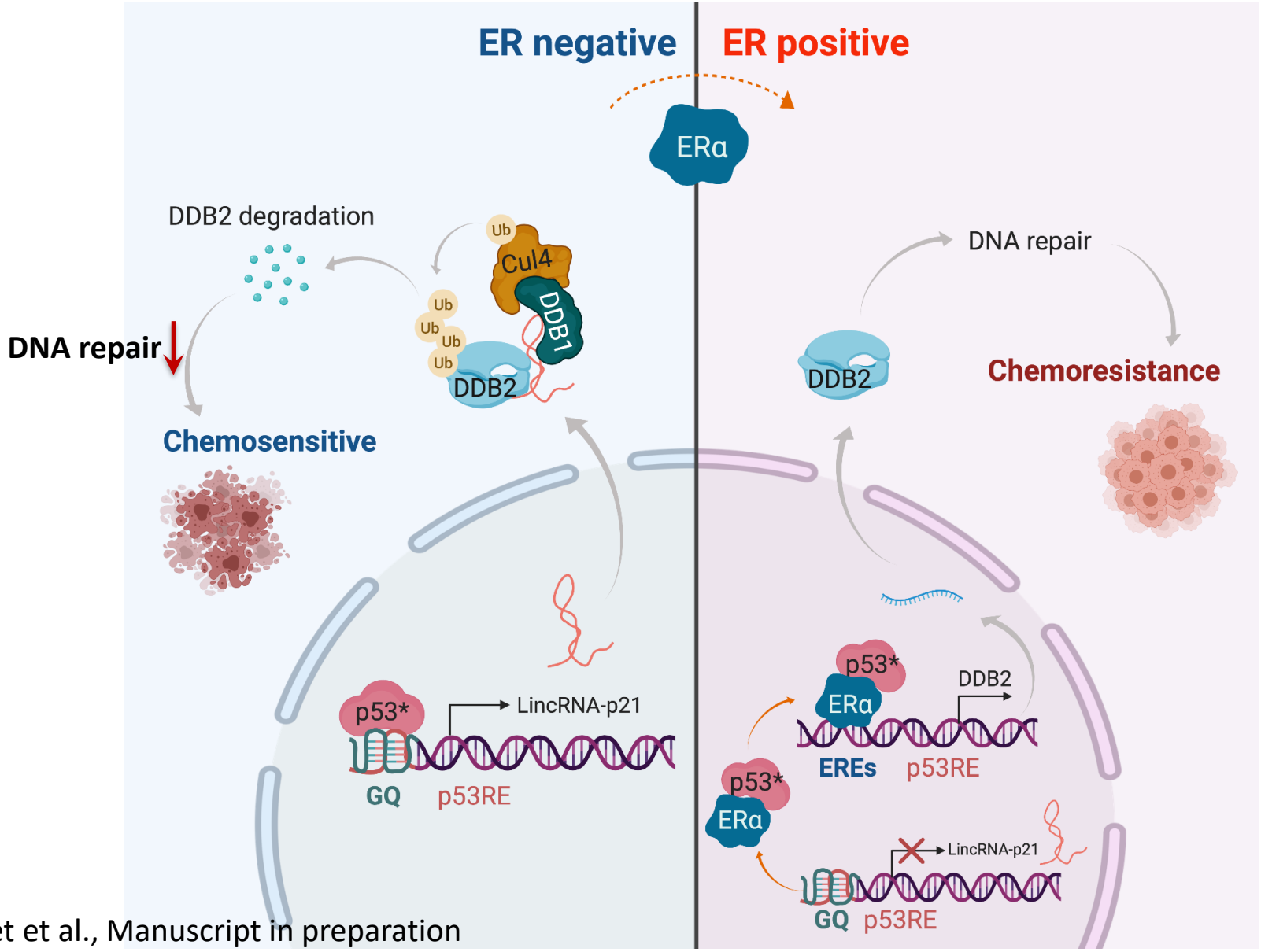




In vitro RNA digestion

He et et al., Manuscript in preparation

LincRNA-p21 acts as an RNA scaffold for DDB2 degradation



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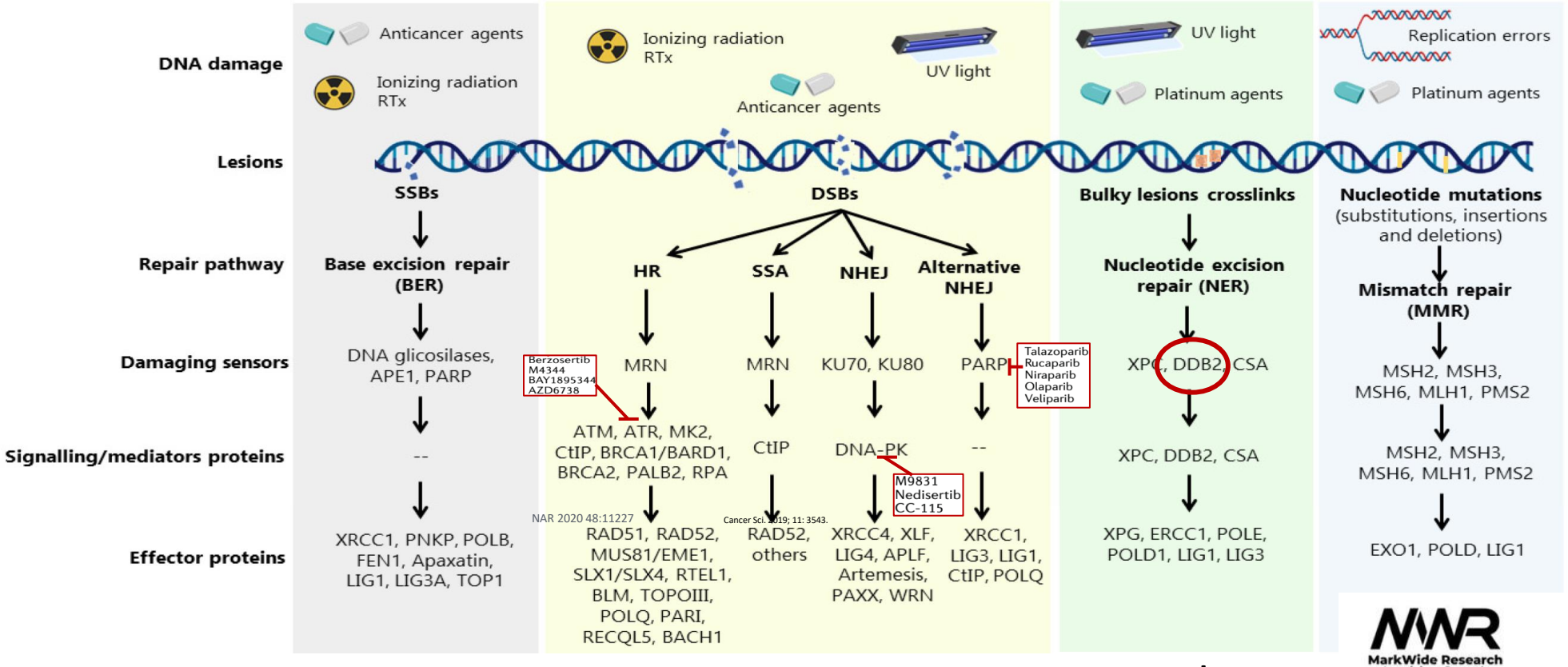
Q4 :

Can lincRNA-p21 be developed as an RNA-based DDB2 degrader?

Unmet Need in DNA Damage Response



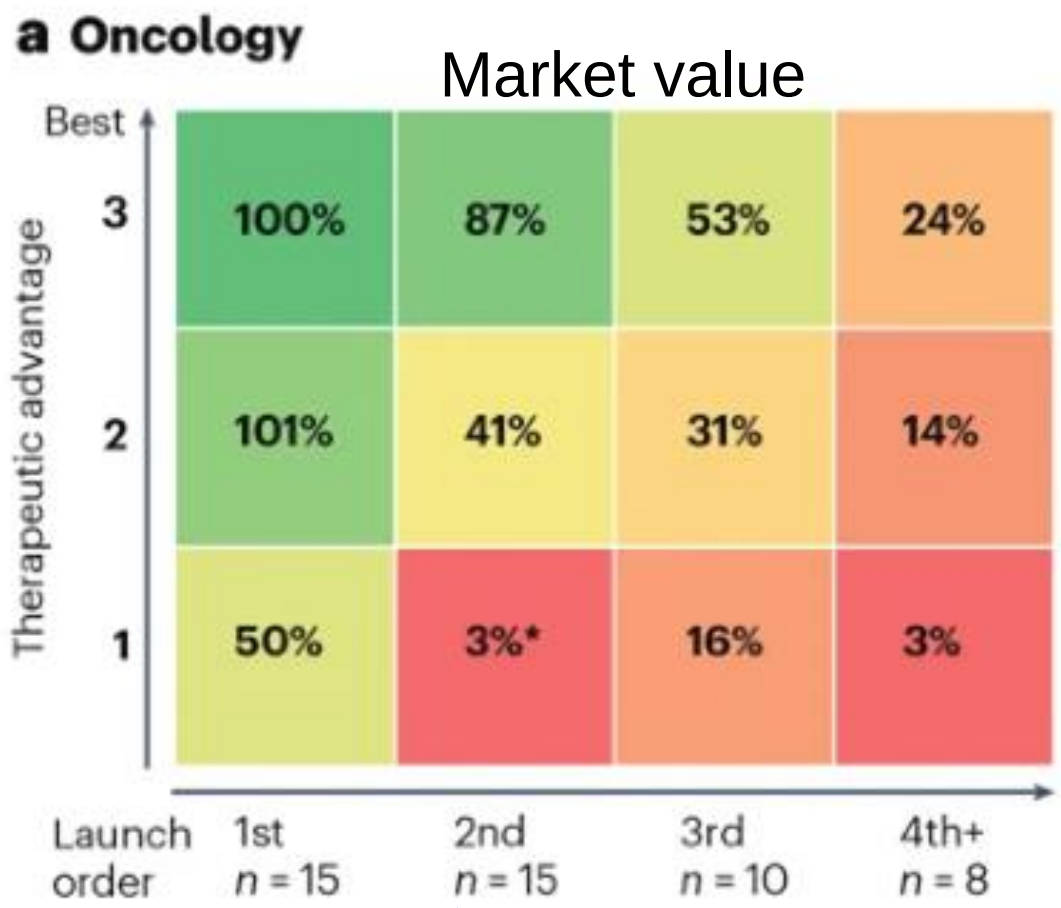
No drug

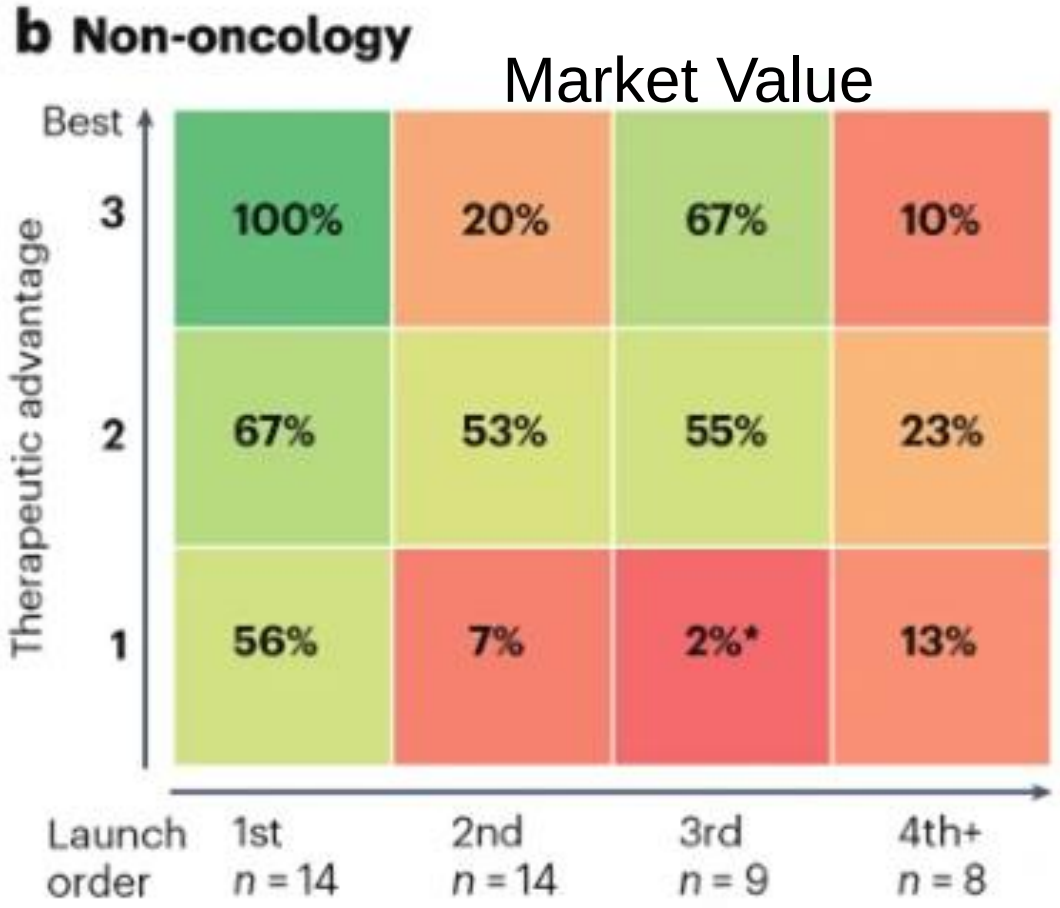
MarkWide Research
Inspiring Growth

DNA Repair Drugs market Key Players

1.AstraZeneca PLC
2.Pfizer Inc.
3.Merck & Co., Inc.
4.Novartis AG
5.GlaxoSmithKline plc
6.Bristol Myers Squibb Company
7.F. Hoffmann-La Roche Ltd.
8.Johnson & Johnson
9.Sanofi S.A.
10.Eli Lilly and Company

First-in-Class Oncology drugs have higher market value



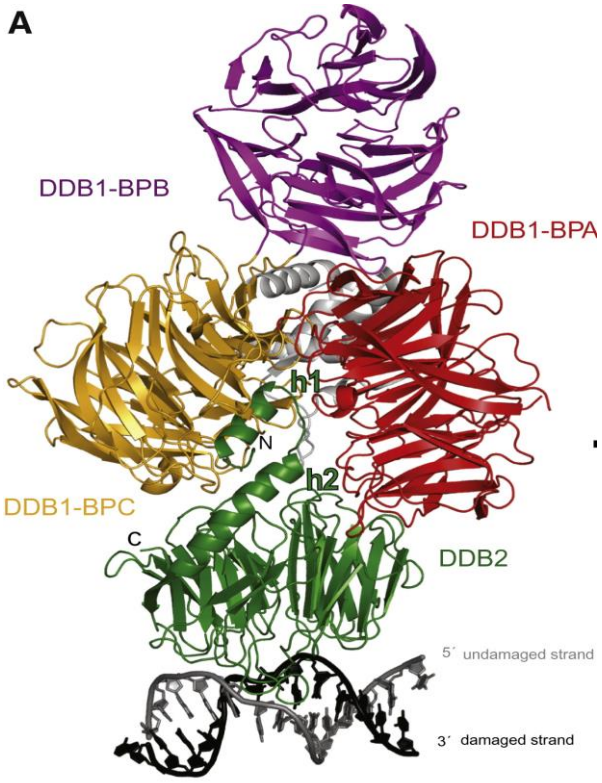


Within 2 year

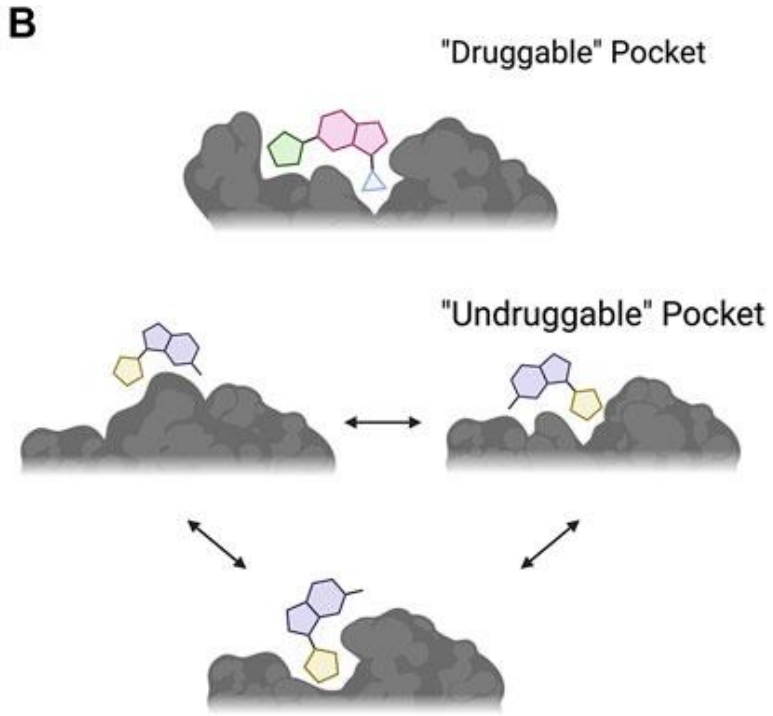
The Boston Consulting Group (BCG)

Nature Reviews Drug Discovery **22**, 531-532 (2023)

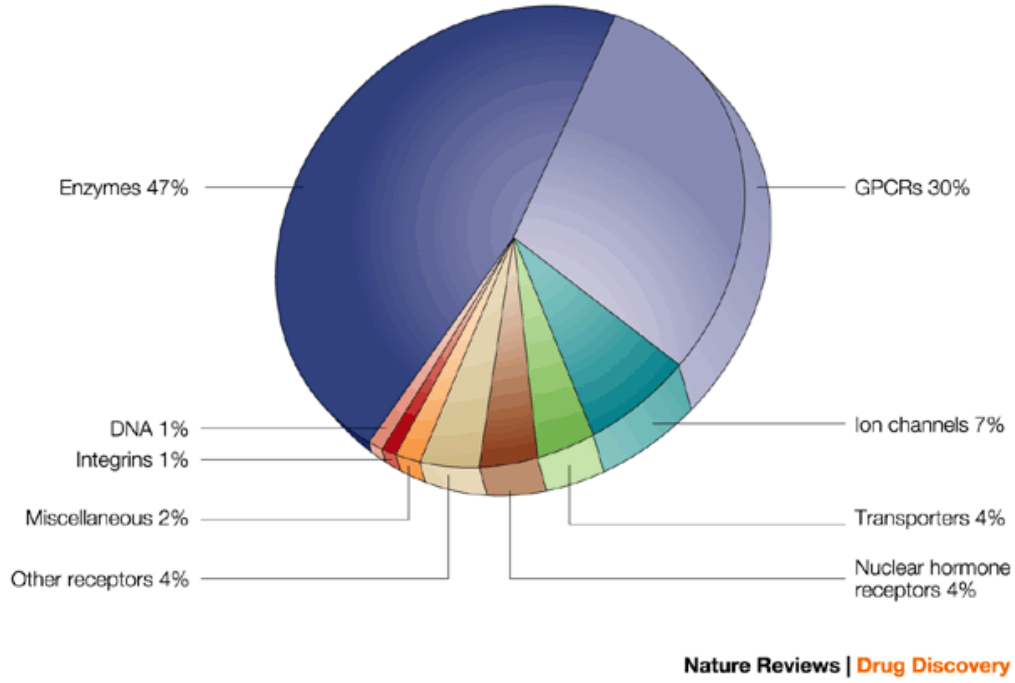
DDB2 is a druggable target?



Cell. 135(7):1213-23, 2008

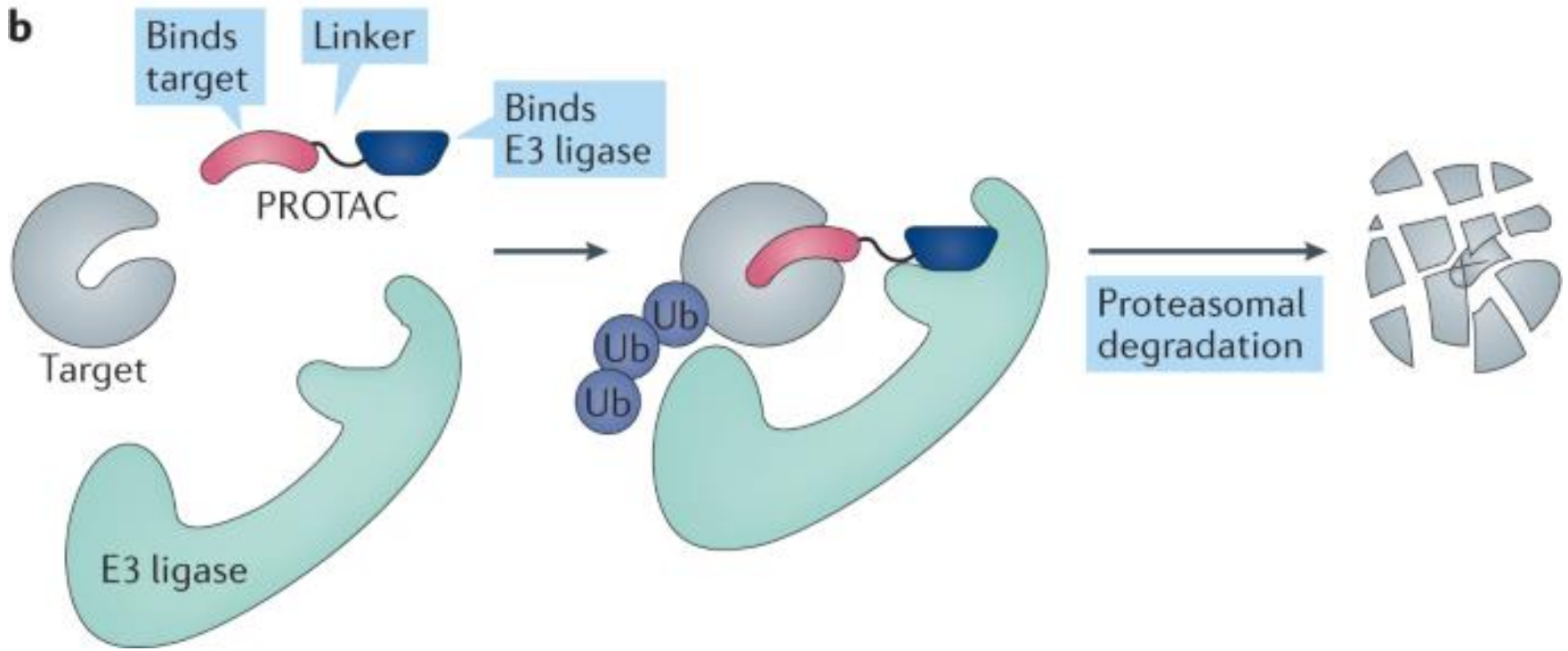


Front. Mol. Biosci., 21 December 2021



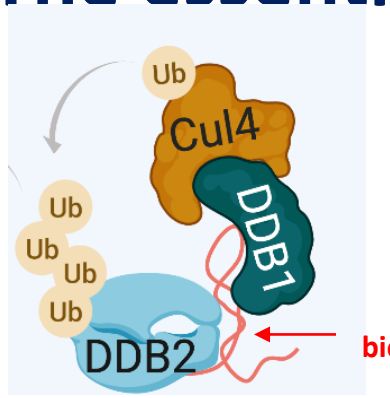
Nature Reviews Drug Discovery **volume 1**, pages 727–730 (2002)

PROTACs (PROteolysis TArgeting Chimeras) make undruggable targets druggable

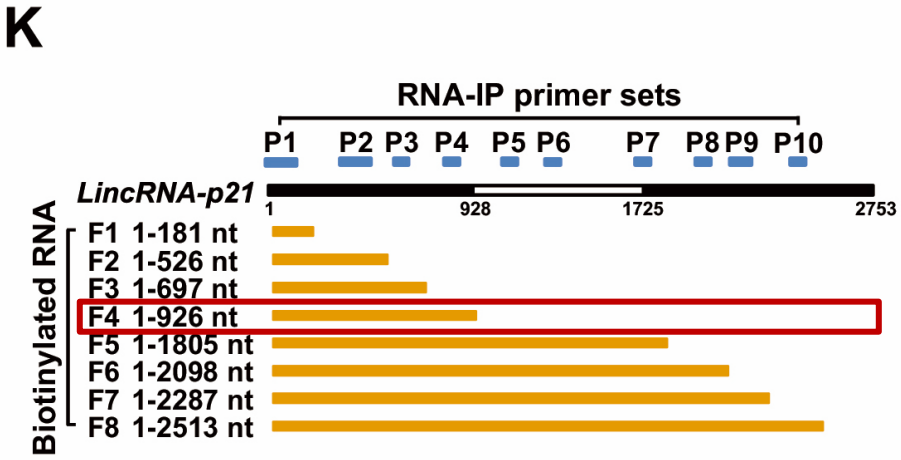


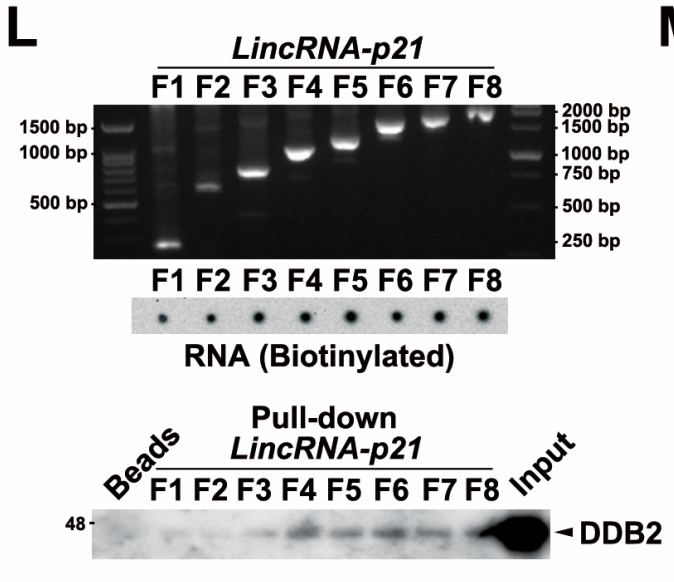
LincRNA-p21 can be developed as a DDB2 degrader like a Protac ?

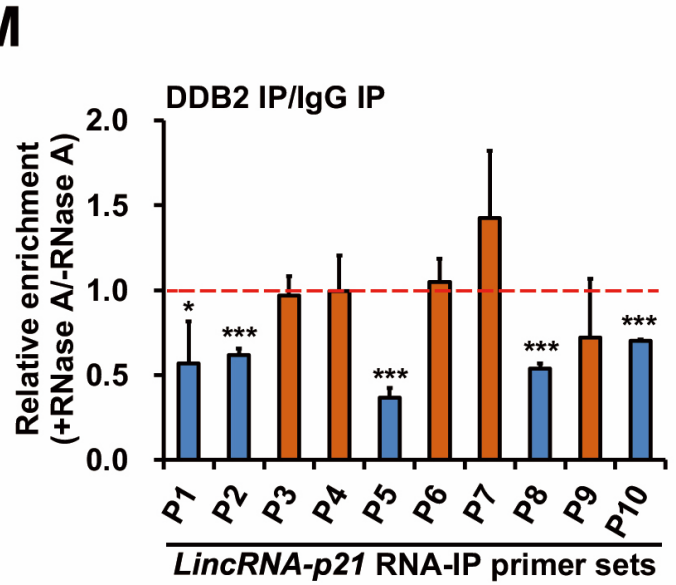
The essential regions of lincRNA-p21 for interactions with DDB2



Biotinylated Probe+ lysate → Pull down with Streptavidin bead → WB for DDB2





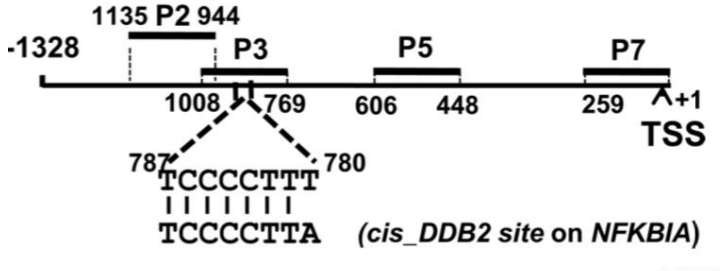


Potential DDB2-binding sites of LincRNA-p21

7838–7849 Nucleic Acids Research, 2015, Vol. 43, No. 16 Published online 29 June 2015
doi: 10.1093/nar/gkv667

DDB2 modulates TGF-β signal transduction in human ovarian cancer cells by downregulating NEDD4L

Ran Zhao[1,†], Tiantian Cui[1,†], Chunhua Han[1], Xiaoli Zhang[2], Jinshan He[1], Amit Kumar Srivastava[1], Jianhua Yu[3], Altaf A. Wani[1,3] and Qi-En Wang[1,3,*]

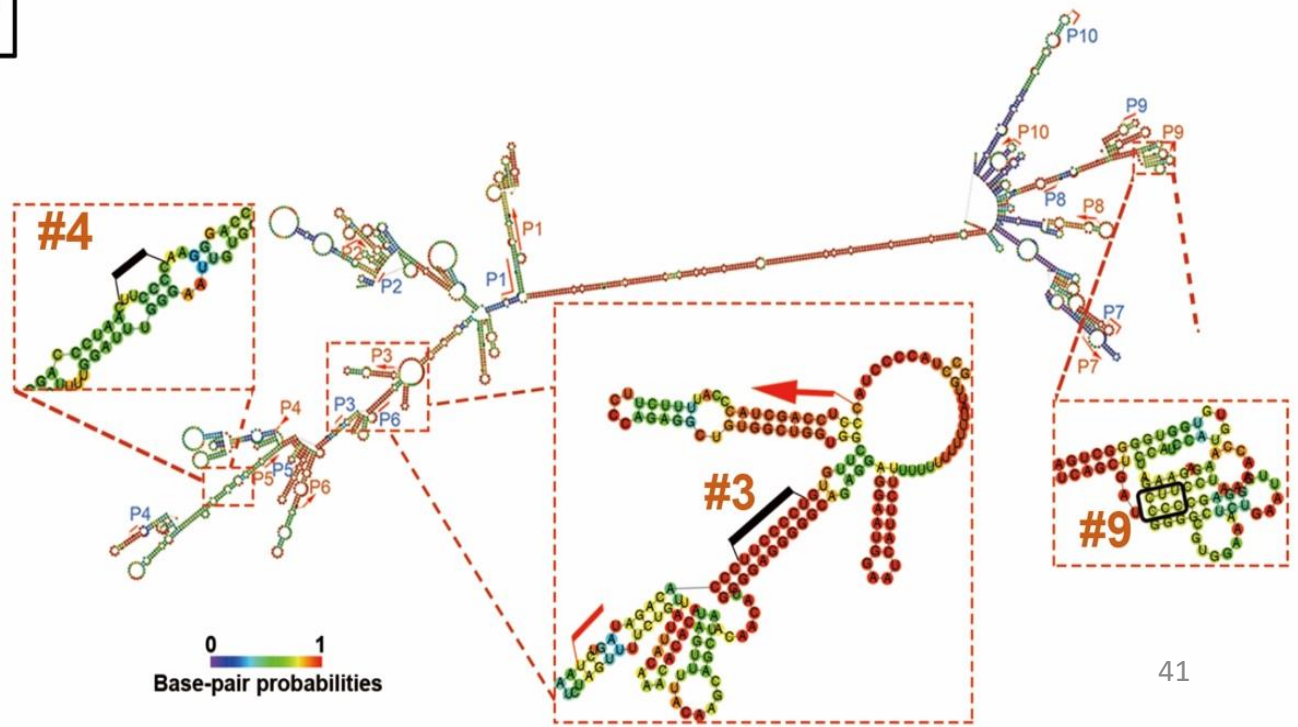


N

LincRNA-p21 secondary structure
(Chr6: 36667296-4544)

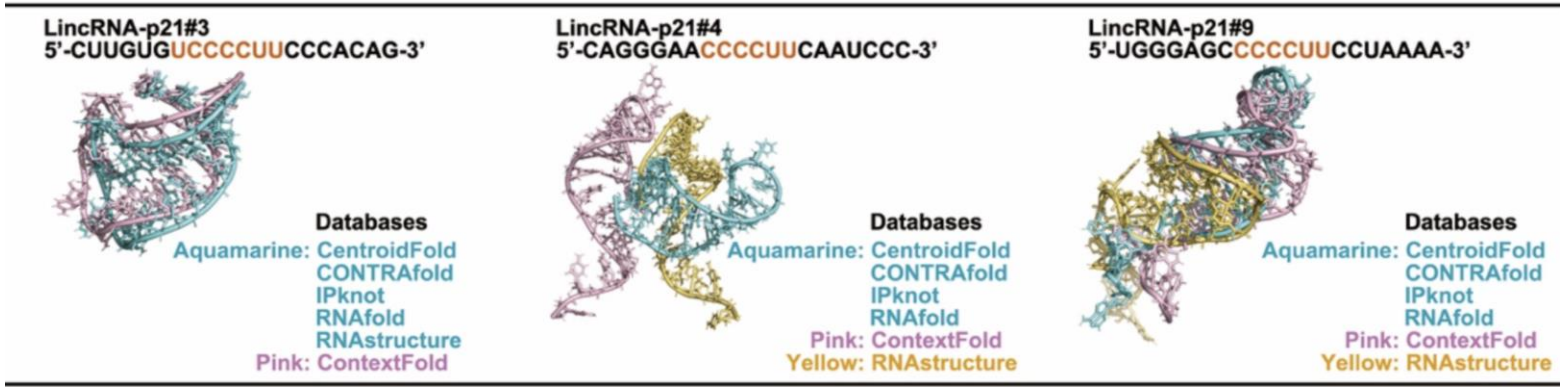
DDB2 binding element: TCCCCTT

- LincRNA-p21#3: UCCCCUU
- LincRNA-p21#4: ACCCCUU
- LincRNA-p21#9: CCCCCUU

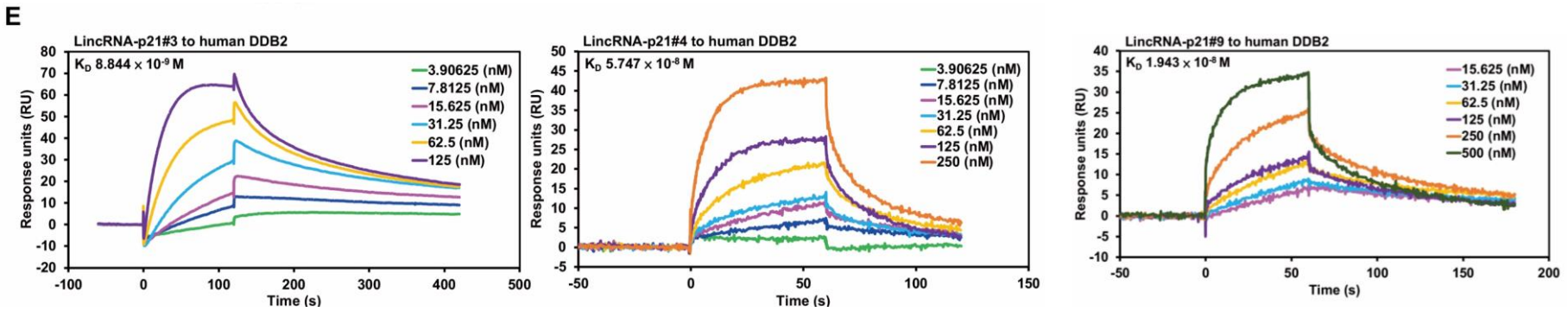


LincRNA-p21 #3, #4, and #9 (Linc-p21s) interact with DDB2 protein *in vitro*

Linc-p21s



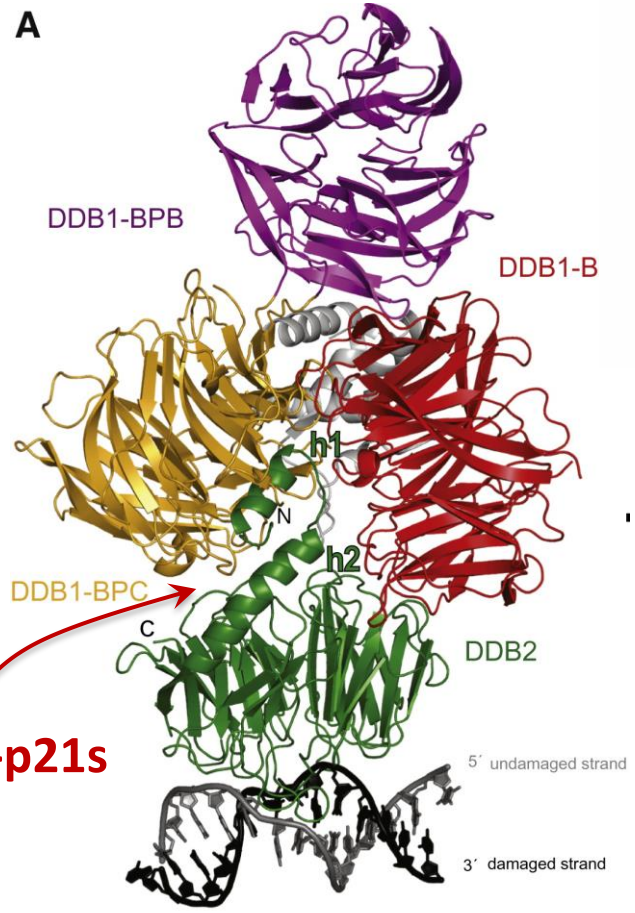
In vitro SPR binding assay



SPR: surface plasmon resonance

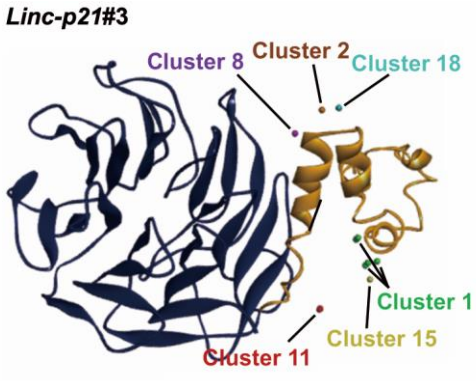
He et et al., Manuscript in preparation

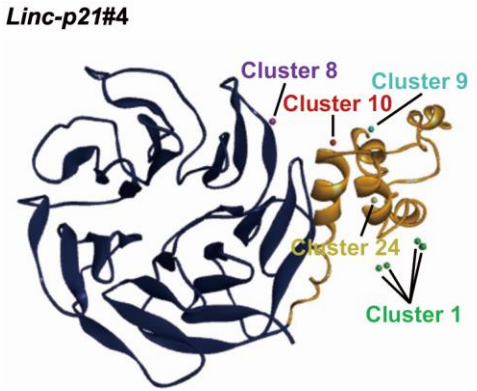
The 3D structural modeling of short lincRNA-p21 elements in complex with the N-terminal α-helix of DDB2

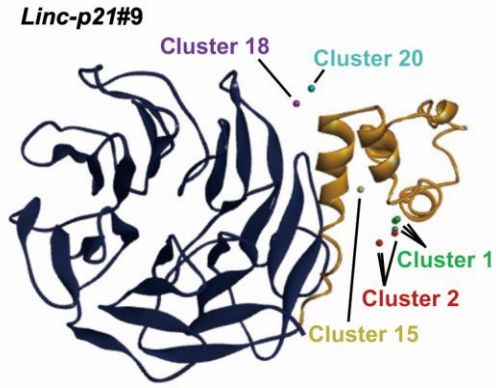


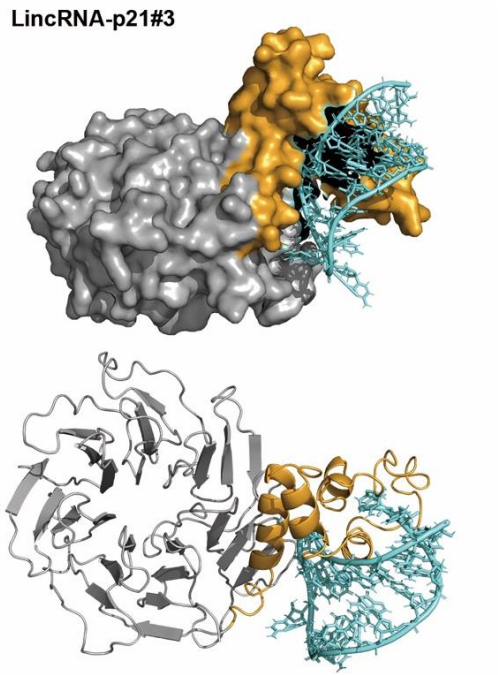
Linc-p21s

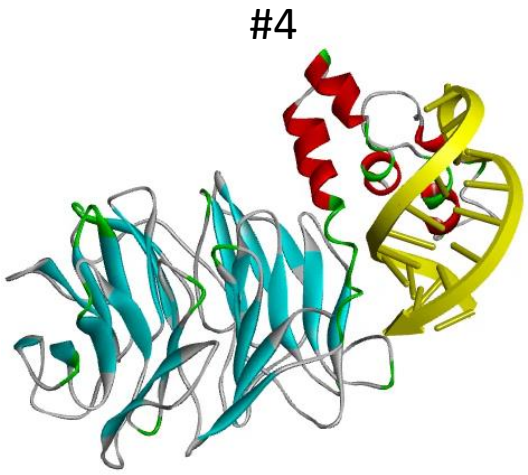
Cell. 135(7):1213-23, 2008







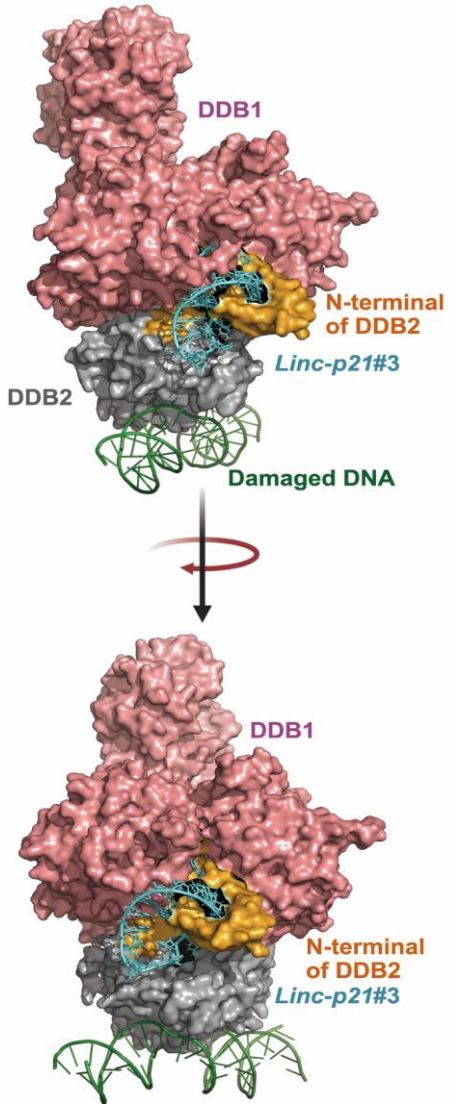


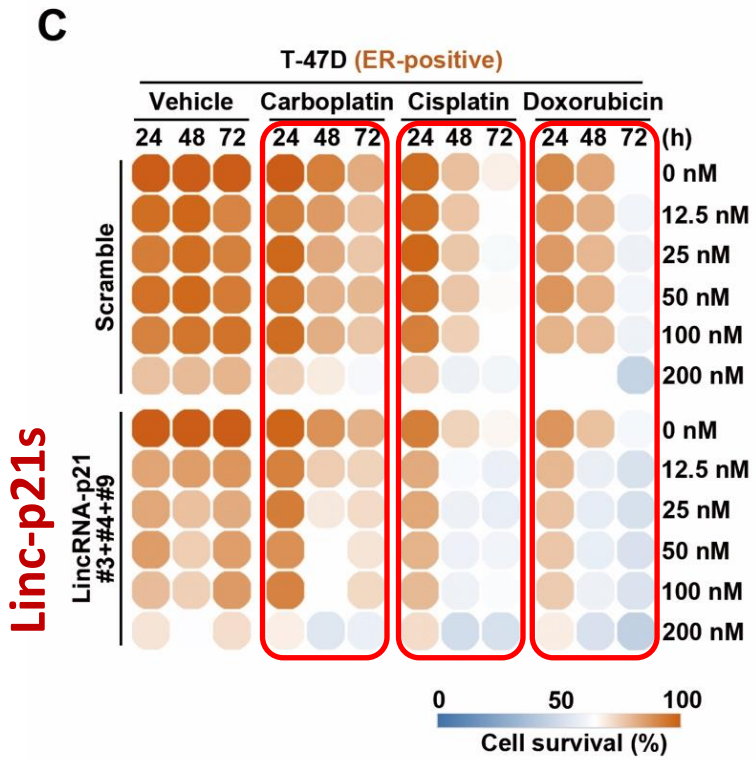


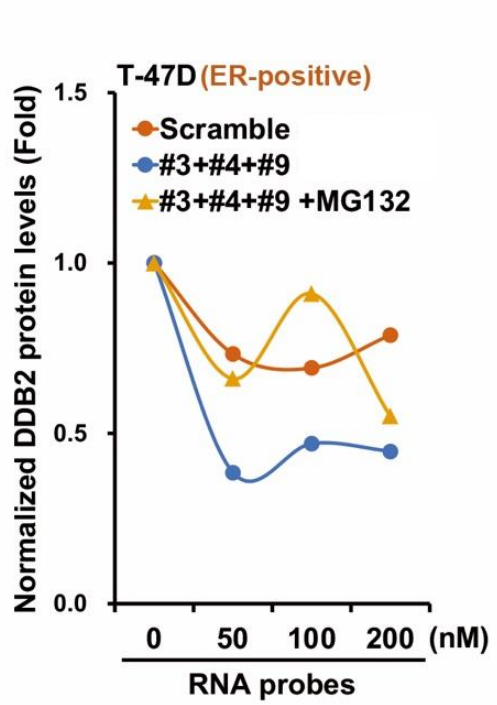
He et et al., Manuscript in preparation

Molecular simulation with Discovery studio

Short lincRNA-p21 elements enhance chemosensitivity







Linc-p21s
Short lincRNA-p21 elements (#3+#4+#9)

↓

DDB2 protein ↓

↓

Chemosensitivity ↑

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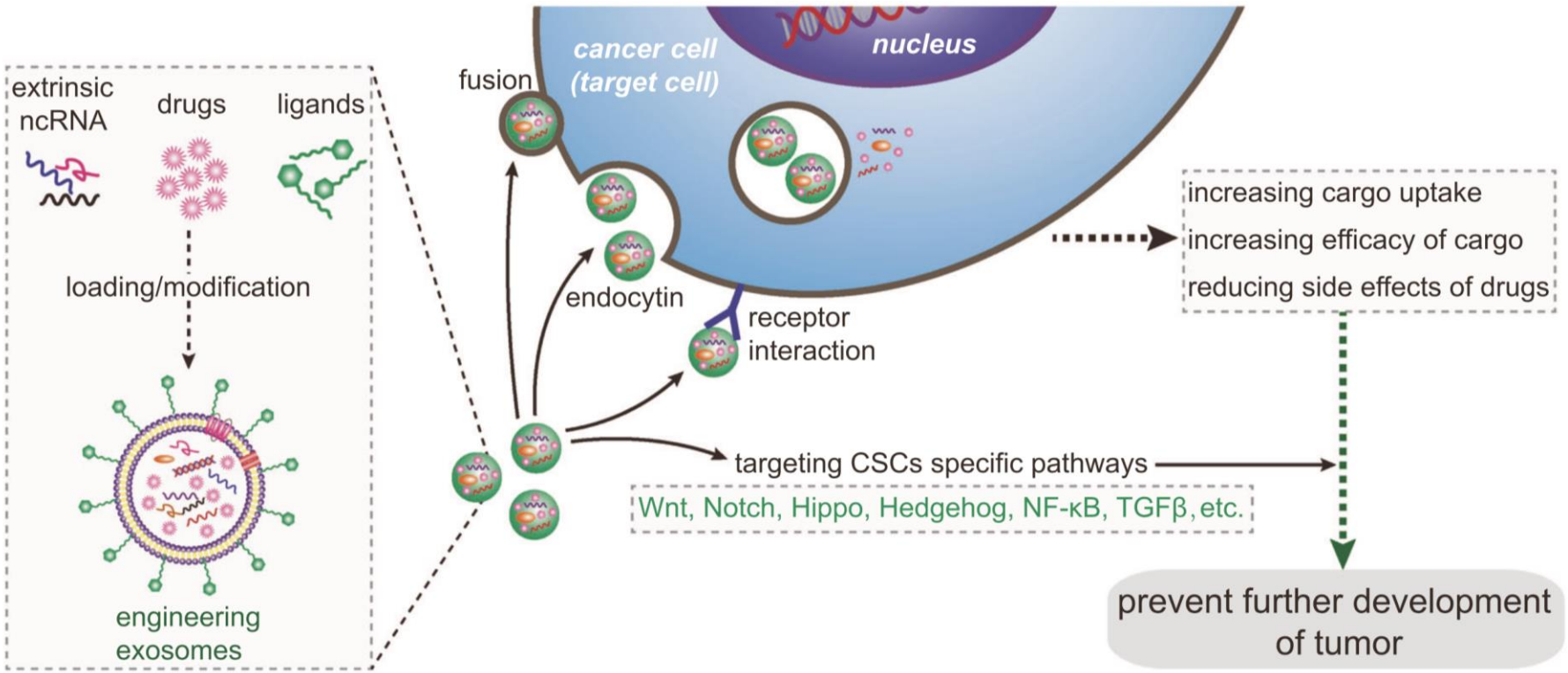
Targeting tumor with engineering exosomes as delivery carrier.



『再生醫療製劑條例』:再生醫療製劑包含細胞治療製劑，可將細胞或其衍生物加工製造，以治療、預防或診斷疾病之製劑

Signal Transduction and Targeted Therapy (2020)5:145

The delivery efficacy of exosomes to T-47D breast cancer cells

Exosome was isolated from HEK-293T cells

Linc-p21s+Doxorubicin+exosome

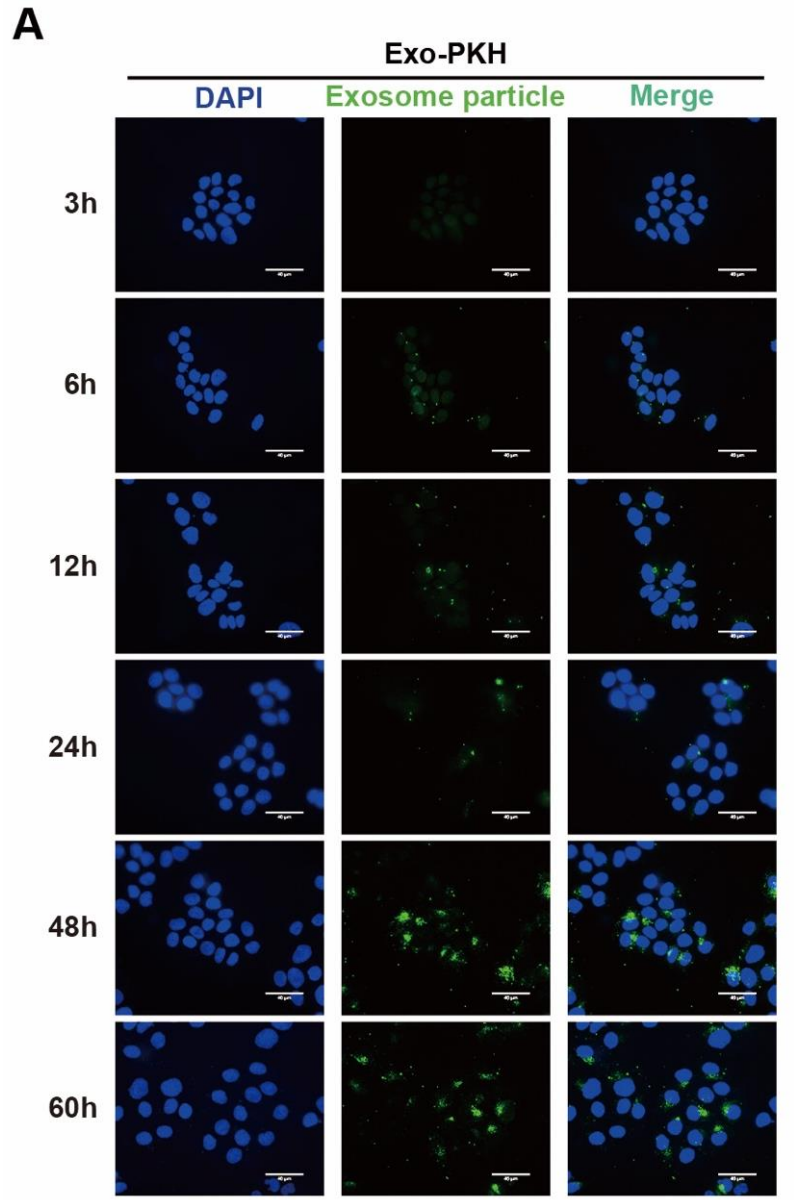
↓

Exo-Linc-p21s

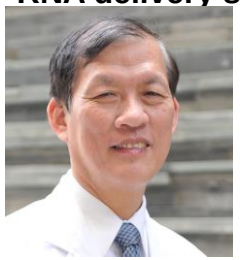
↓ ?

Sensitize to chemo-drugs

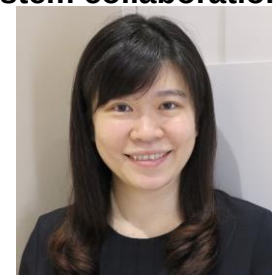
PKH: lipophilic membrane dye for exosome



RNA delivery system collaboration



周德陽院長

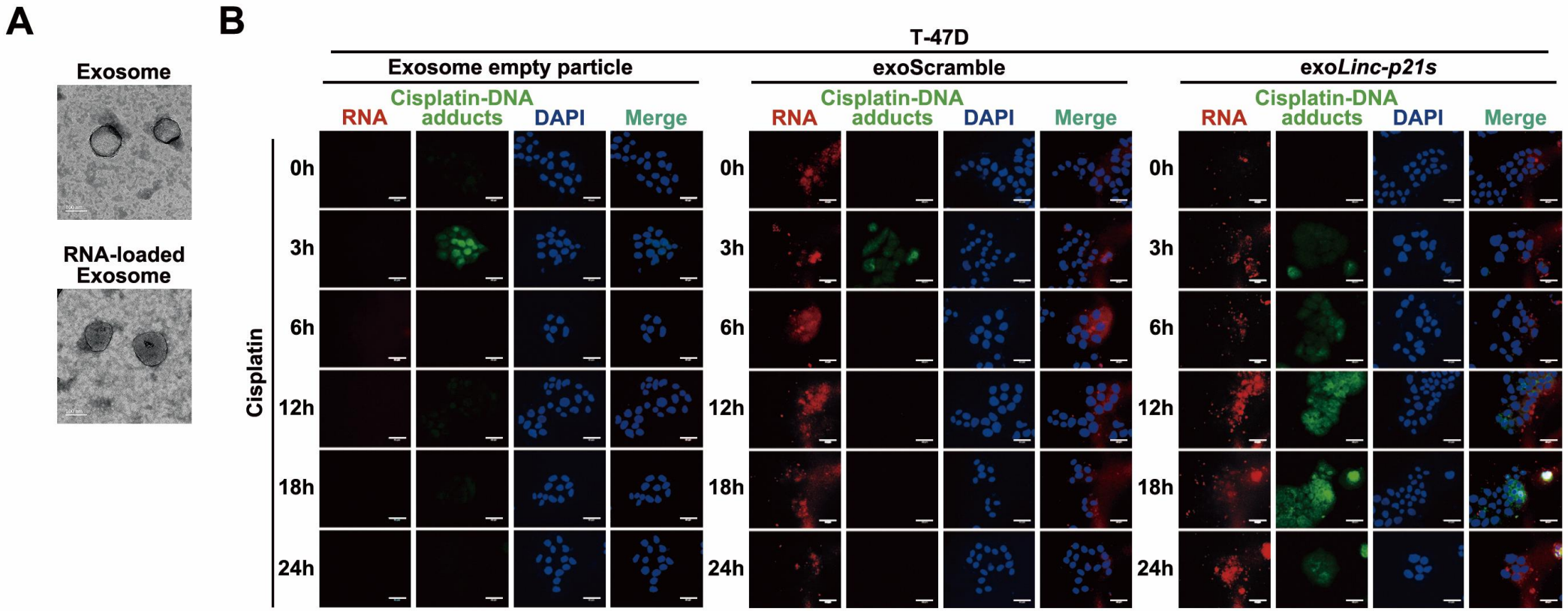


陳怡文教授

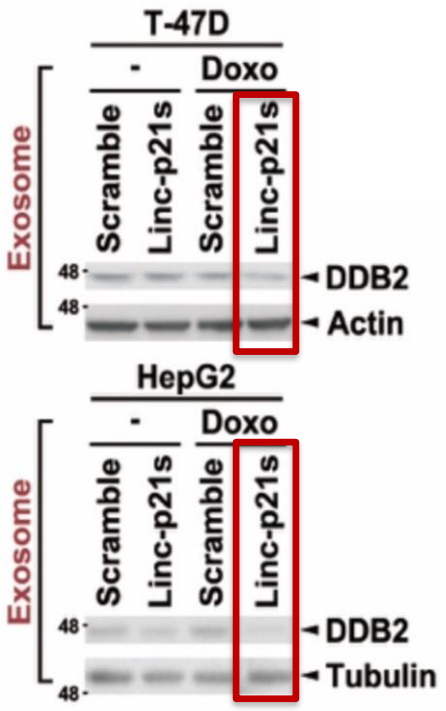
謝明佑教授

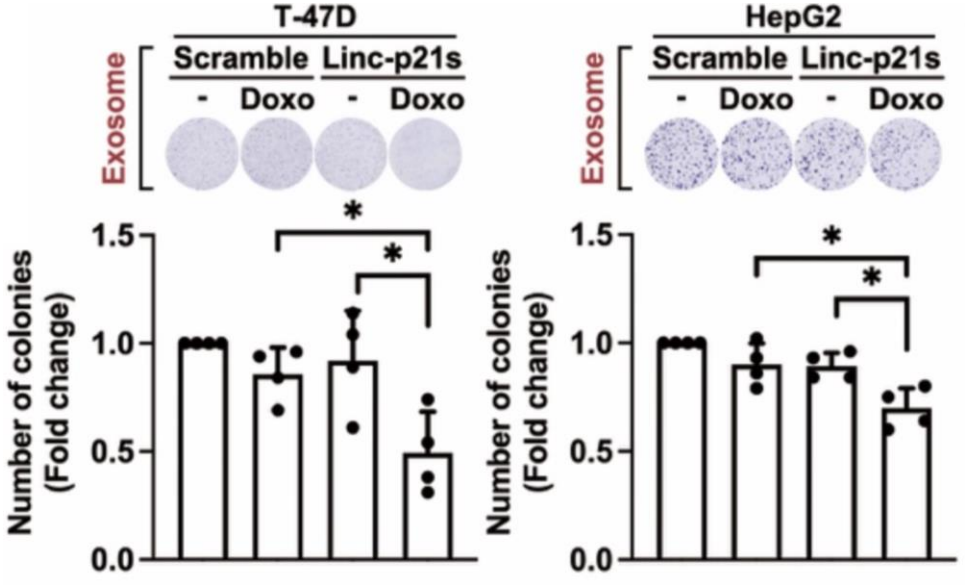
x-Dimension Center for Medical Research and Translation,
China Medical University Hospital,
Taichung, Taiwan

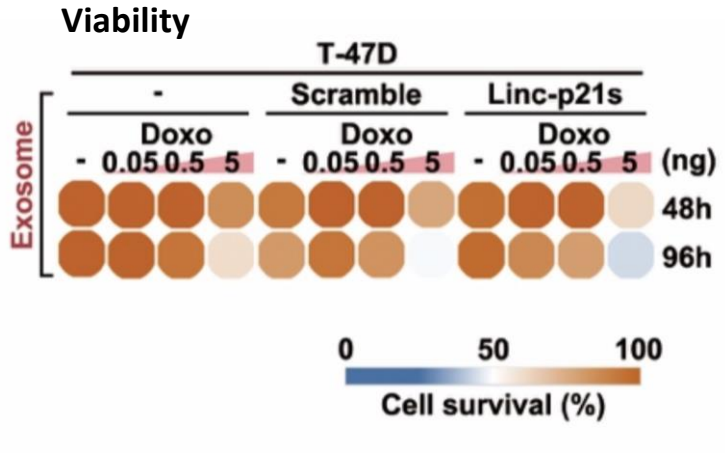
Exo-Linc-p21s reduce DNA repair

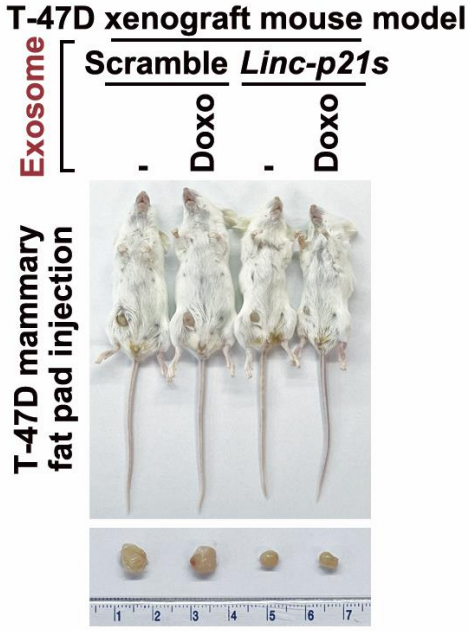


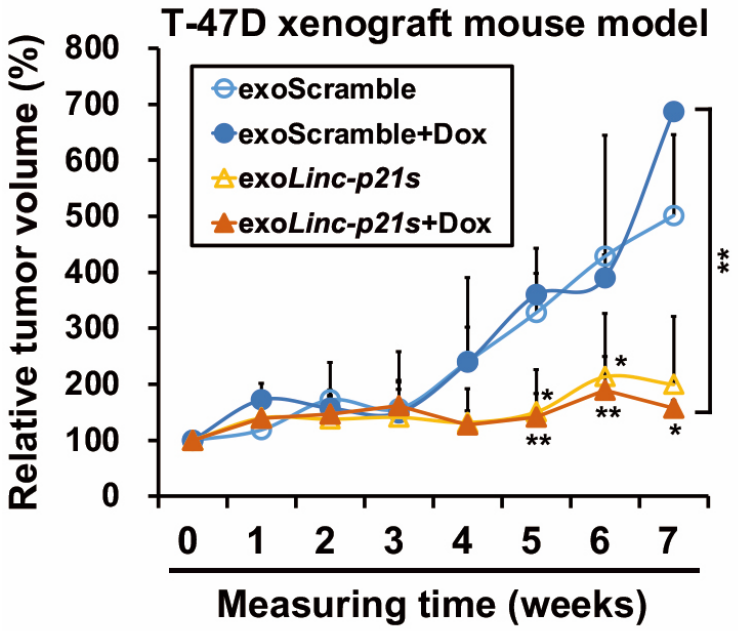
Exo-Linc-p21s increase the chemosensitivity of breast and liver cancer cells



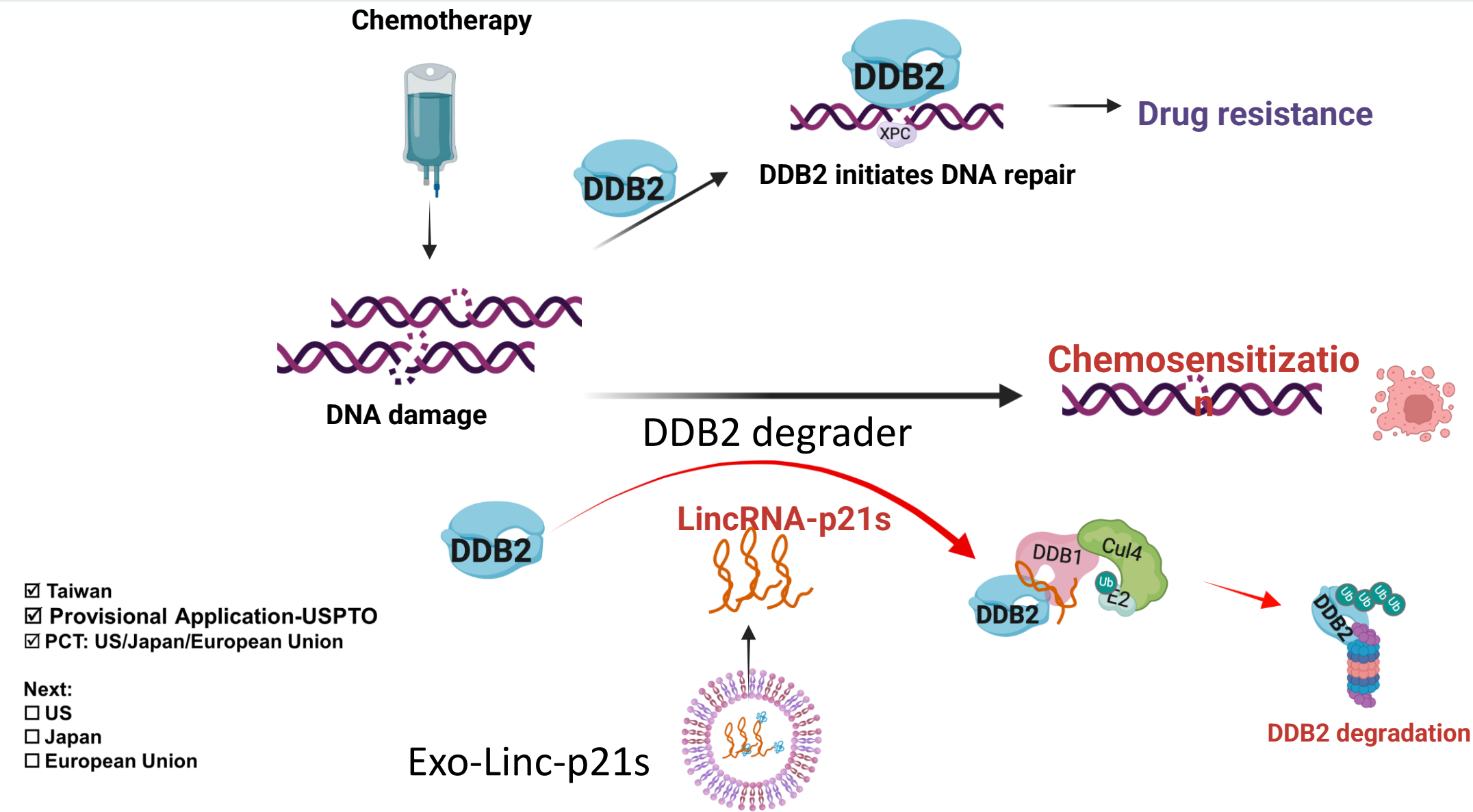








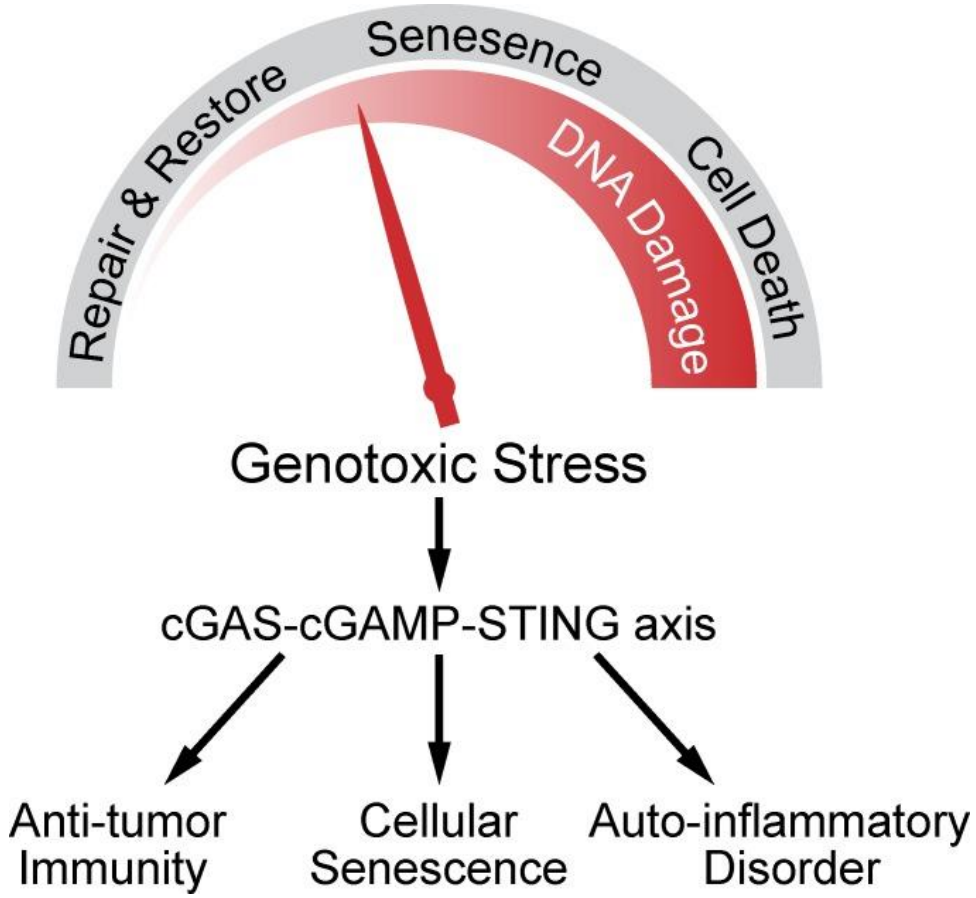
Exo-linc-p21s is a Novel Molecular Glue Targeting DDB2 and Enhancing Chemotherapy Sensitivity



☑ Taiwan
☑ Provisional Application-USPTO
☑ PCT: US/Japan/European Union

Next:
☐ US
☐ Japan
☐ European Union

The cGAS–cGAMP–STING pathway connects DNA damage to inflammation, senescence, and cancer



J Exp Med. 2018 May 7; 215(5): 1287–1299.

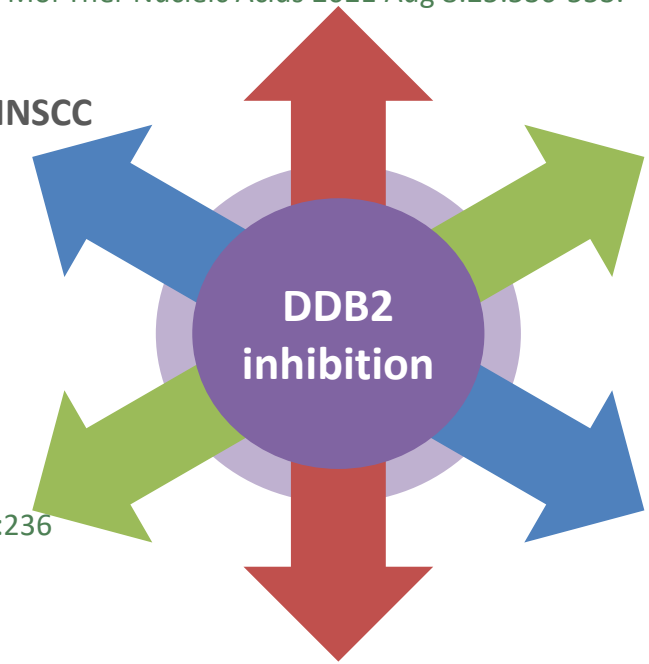
Therapeutic benefits from targeting DDB2

Topo II inhibitor (Anthracyclin) sensitization in p53-deficient breast cancer

Mol Ther Nucleic Acids 2021 Aug 8:25:536-553.

Overcome cisplatin resistance in HNSCC

Cell Death Dis. 2022 Apr 15;13(4):350



Sensitize TNBC and PDAC to PARPi

Cancer Sci. 2019 Nov;110(11):3543-3552.
Cell Death Discov. 2024 Sep 27;10(1):411.

Increase sensitivity to ADC?

J Exp Clin Cancer 2024 Aug 21:43(1):236
J Clin Invest. 2023;133(18):e172156

Increase radiosensitivity in NSCLC and PDAC

Tumour Biol. 2016 Oct;37(10):14183-14191
Cell Death Discov. 2024 Sep 27;10(1):411.

Activate cGAS/STING to improve ICI efficacy?

Proteomes. 2020 Oct 26;8(4):30

Acknowledgements

Postdoctoral Fellow
Dr. Yu-Hao He

Huang's lab

Dr. Thanh Kieu Huynh
Dr. Fang-Ju Cheng
Ya-Ling Wei
Shu-Wei Hu
Dai-Wei Hu
Yi-Lun Yeh

Clinical collaboration

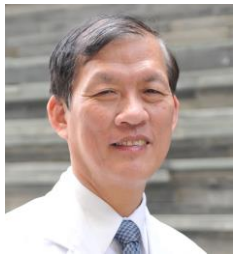

葉名烻主任

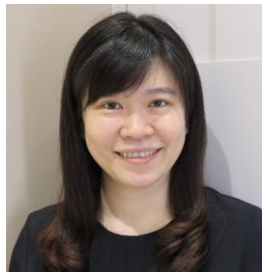

劉良智主任


黃至豪醫師

CMUH & CSUH

RNA delivery system collaboration


周德陽院長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Thank you for your attention