Molecular and Genetic Biomarkers of Tumor Response to Radiation

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Workshop – Individual Response to Ionizing Radiation 1-2 September 2022, Stockholm, Sweden

Radiation response of tumors

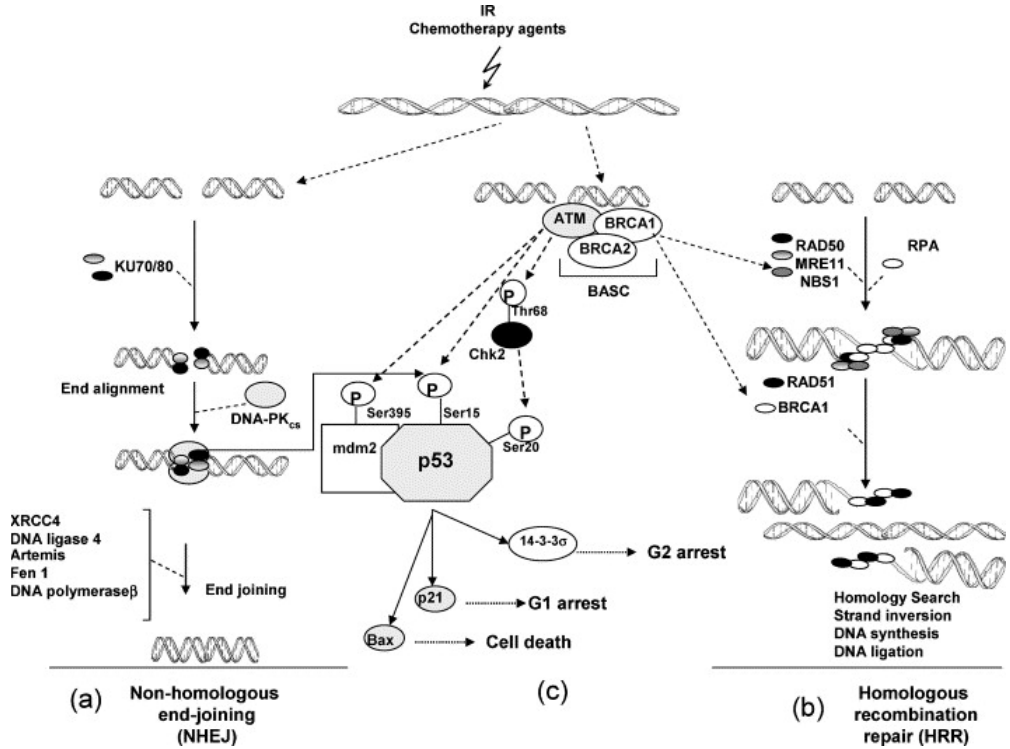
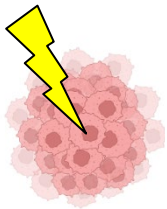
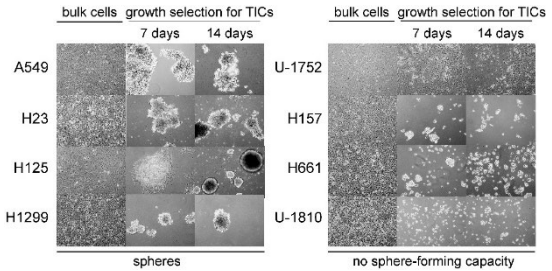


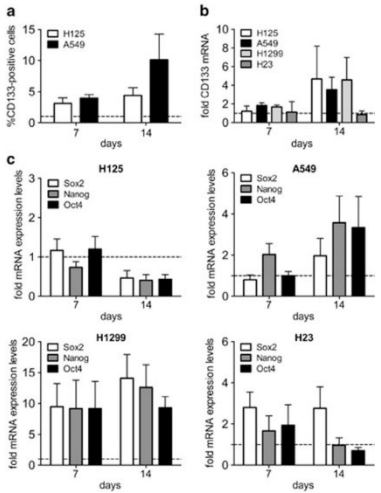
Fig.1

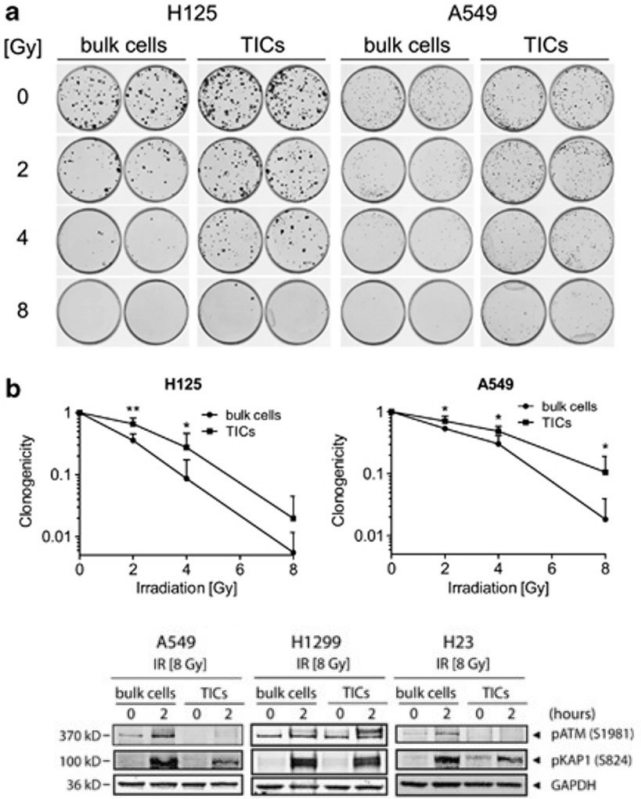
Viktorsson K, De Petris L, Lewensohn R. The role of p53 in treatment responses of lung cancer. Biochem Biophys Res Commun. 2005 Jun 10;331(3):868-80. doi: 10.1016/j.bbrc.2005.03.192. PMID: 15865943.

Resistance to Ionizing radiation in Tumor initiating cells – altered DNA repair function



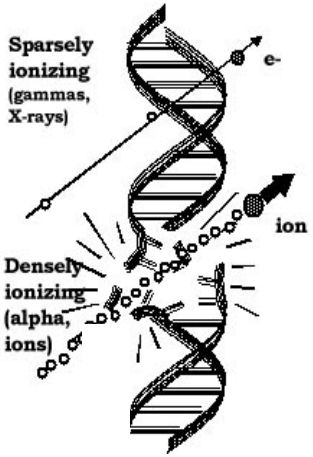


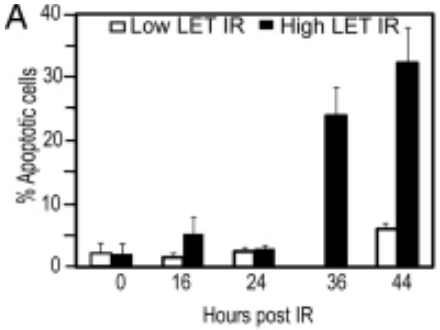


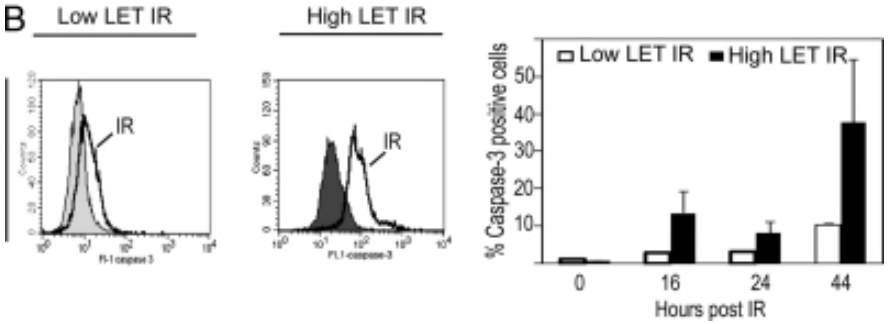


Ref: Lundholm L, Hååg P, Zong D, Juntti T, Mörk B, Lewensohn R, Viktorsson K. Resistance to DNA-damaging treatment in non-small cell lung cancer tumor-initiating cells involves reduced DNA-PK/ATM activation and diminished cell cycle arrest. Cell Death Dis. 2013 Jan 31;4(1):e478.

Radiation response in tumor cells depends on radiation quality

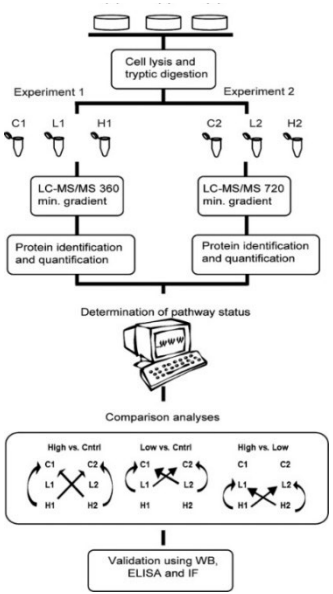


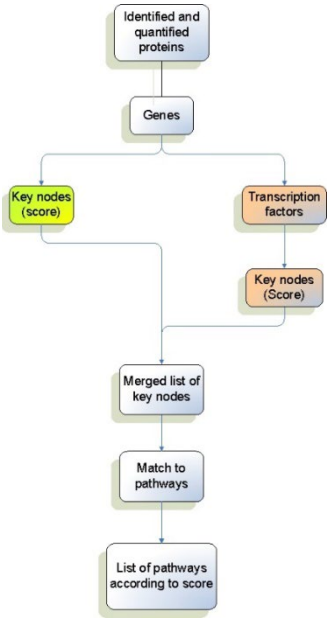




Ref: Ståhl S, Fung E, Adams C, Lengqvist J, Mörk B, Stenerlöw B, Lewensohn R, Lehtiö J, Zubarev R, Viktorsson K. Proteomics and pathway analysis identifies JNK signaling as critical for high linear energy transfer radiation-induced apoptosis in non-small lung cancer cells. Mol Cell Proteomics. 2009 May;8(5):1117-29.

MS-based protemics to identify signaling differences-identification of JNK-driven apoptotic signaling





	Activated pathways	ΔS	Deactivated pathways	ΔS	
Top 3	**JNK pathway ***	**24897**	**p38 pathway**	**-3561**	Top 3
	Fas pathway	**2929**	**RANKL pathway**	**-1897**	
	EGF pathway	**2625**	**Epo pathway**	**-1713**	
	JNK1 ---> MKK4	1302	PRL pathway	-633	
	IL-8 pathway	175	BMP2 ---> p38alpha	-519	
	Neurotensin pathway	151	TAK1 ---> ATF-2	-287	
	Caspase network	127	EGF ---> PAK1	-185	
	LAT ---> PKCbeta	86	Apo2L pathway	-159	
	Two different pathways	77	RANKL ---> MITF	-130	
	Three different pathways	36	Epo ---> ERK1	-88	
	Two different pathways	27	TGFbeta pathway	-74	
	wnt pathway	25	ZAP-70 ---> c-Jun	-50	
	Seven different pathways	5	Two different pathways	-40	
			Five different pathways	-33	
			Three different pathways	-25	
			25 different pathways	-14	
			197 different pathways	-4	

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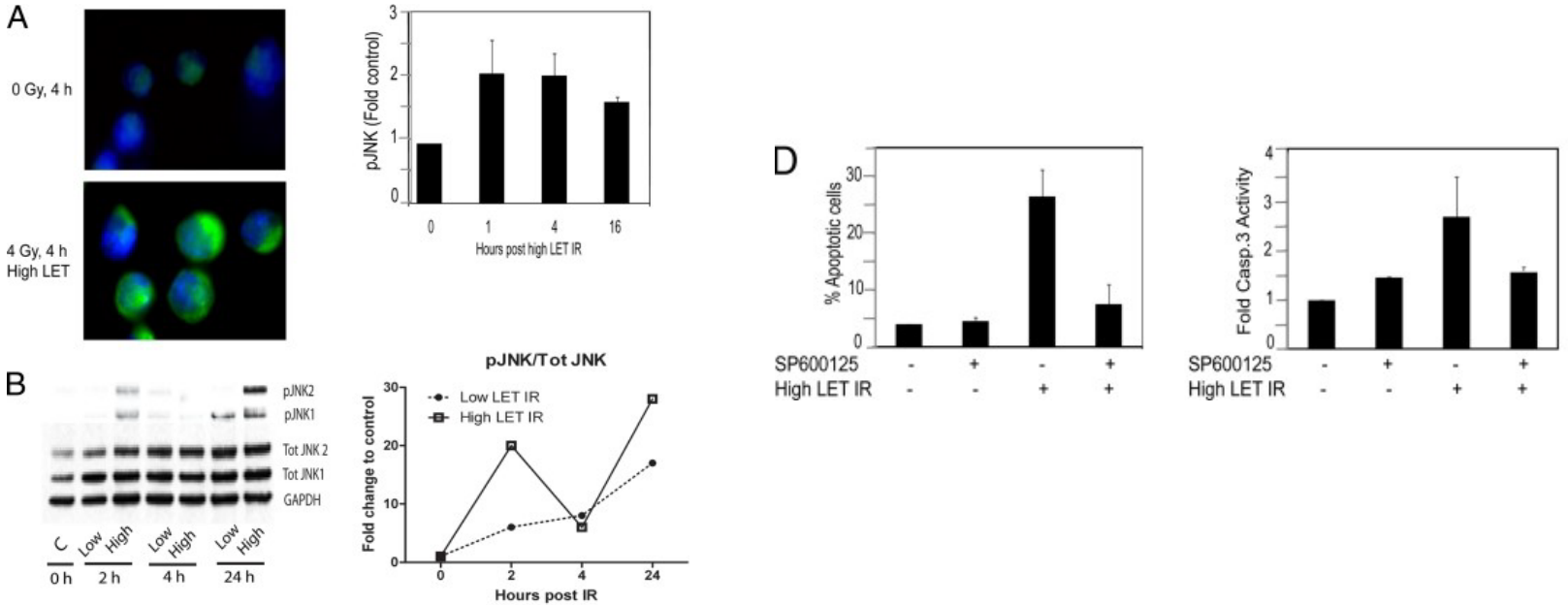

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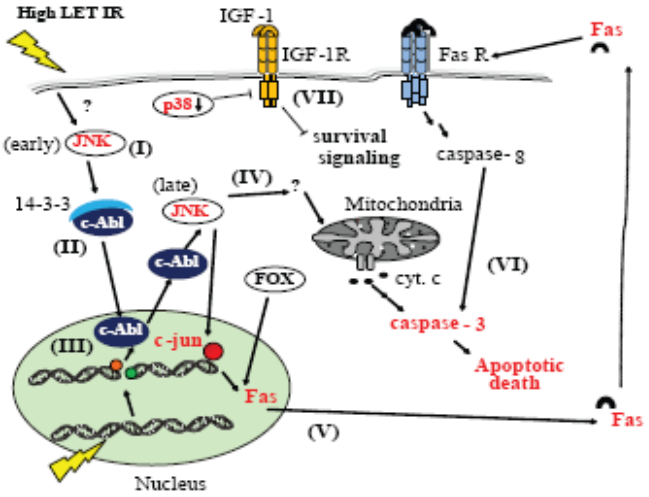
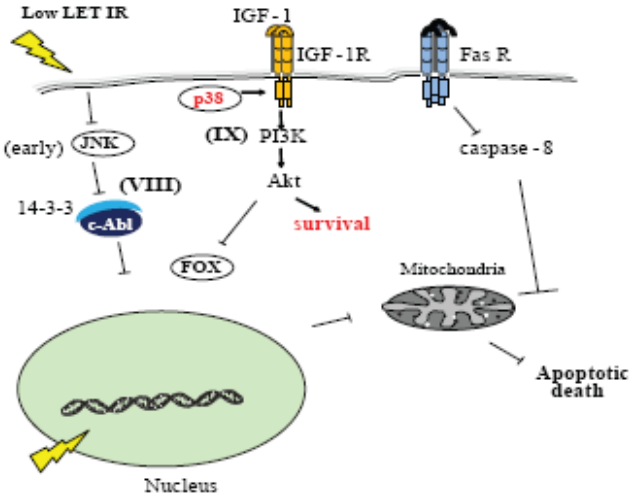
Conclusion: Radiation-induced DNA damaging signaling depends on radiation quality and influence apoptotic propensity





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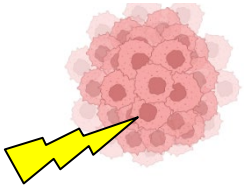
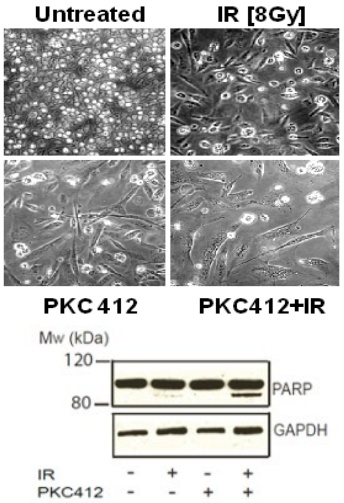
Gene expression analyses of IR-resistance pathways in Non-small cell lung cancer cells indentifies Ephrin B3



+RO-31-8220 → Survival

+PKC 412 → Cell death

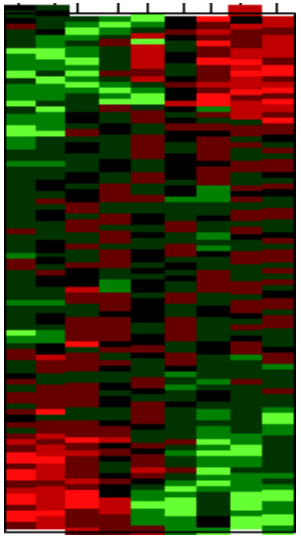
Genexpression analyses



↓	↑	↓	↑
MAS1	SRC	SGK	RASSF4
WNT16	**RAB33A**	GADD45B	PPP2R2C
Ephrin B3	ATM	JUNB	ARHI
JUND	FOSB	RUNX1	GNAO1
NOTCH1	ARNT	**RAB33A**	BCL2
FOXC1	RAB7L1	SPRY2	ADAM18
PRKCA	PACAP	FGFR2	**ESR1**
ESR1		APAF1	BCL2
PP2RC		CASP8AP2	
BAG4		**CDH6**	
PACAP		**TGFB1/4**	

IR+PKC 412

IR+RO-31-8220



Ref: Ståhl S, Kaminskyy VO, Efazat G, Hyrslova Vaculova A, Rodriguez-Nieto S, Moshfegh A, Lewensohn R, Viktorsson K, Zhivotovsky B. Inhibition of Ephrin B3-mediated survival signaling contributes to increased cell death response of non-small cell lung carcinoma cells after combined treatment with ionizing radiation and PKC 412. Cell Death Dis. 2013 Jan 10;4(1):e454..

The Ephrin & Eph signaling circuit-effect on multiple hallmarks of cancer

- Important in cell communication
- Multiple role in normal physiology &disease pathogenesis

Ex. Neuronal system- axon guidance and synaptogenesis.

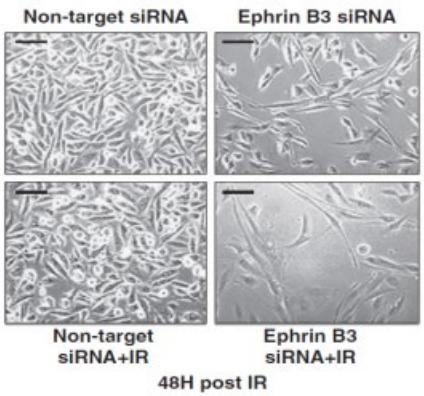
- Linked to cancer early on: **E**rythropoietin-**p**roducing **h**epatocellular carcinoma (**Eph**) receptors

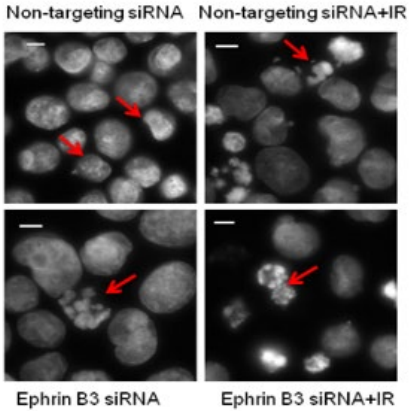
Ex. EphA1 cloned from hepatocarcinoma in a screen for oncogenic tyrosine kinase.

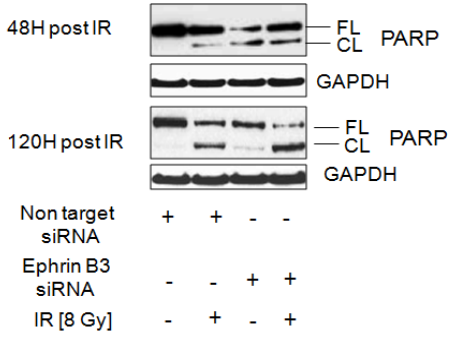
- Multiple Eph receptors and/or Ephrins are present in essentially all types of cancer cells.

For overview of Eph signaling and for the statements above Boyd AW, Bartlett PF, Lackmann M. Therapeutic targeting of EPH receptors and their ligands. Nat Rev Drug Discov. 2014 Jan;13(1):39-62. doi: 10.1038/nrd4175. PMID: 24378802.

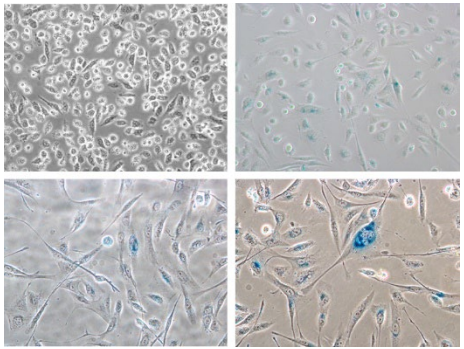
Inhibition of Ephrin B3 sensitizes NSCLC cells to IR



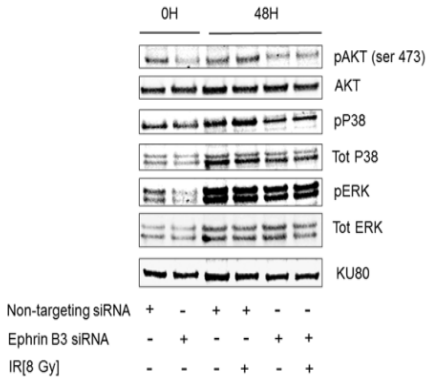




Non-target siRNA Ephrin B3 siRNA



Non-target siRNA+ IR Ephrin B3 siRNA +IR



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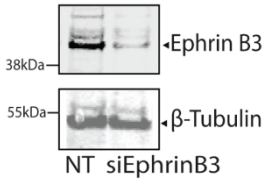
Journal of proteome research

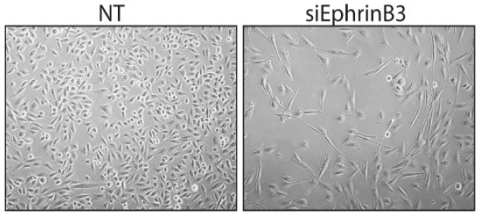
ARTICLE

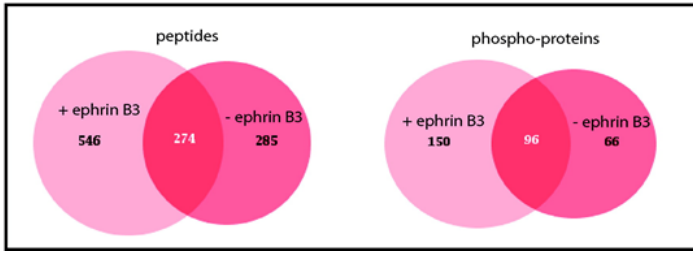
pubs.acs.org/jpr

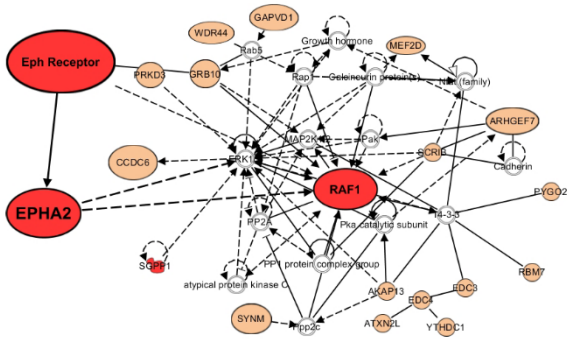
Phosphoproteomic Profiling of NSCLC Cells Reveals that Ephrin B3 Regulates Pro-survival Signaling through Akt1-Mediated Phosphorylation of the EphA2 Receptor

Sara Ståhl,[†] Rui Mm Branca,[†] Ghazal Efazat,[†] Maria Ruzzene,[‡] Boris Zhivotovsky,[§] Rolf Lewensohn,[†] Kristina Viktorsson,[†] and Janne Lehtiö*[,†,∥]







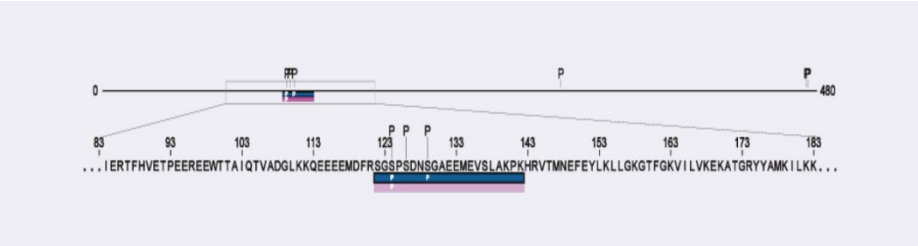


Ref: Ståhl S, Branca RM, Efazat G, Ruzzene M, Zhivotovsky B, Lewensohn R, Viktorsson K, Lehtiö J. Phosphoproteomic profiling of NSCLC cells reveals that ephrin B3 regulates pro-survival signaling through Akt1-mediated phosphorylation of the EphA2 receptor. J Proteome Res. 2011 May 6;10(5):2566-78. doi: 10.1021/pr200037u. Epub 2011 Apr 11. PMID: 21413766.

Silencing of Ephrin B3 alters EphA2 ser897 phosphorylation

In presence of Ephrin B3:

p-EphA2 ser897



p-Akt Ser 129

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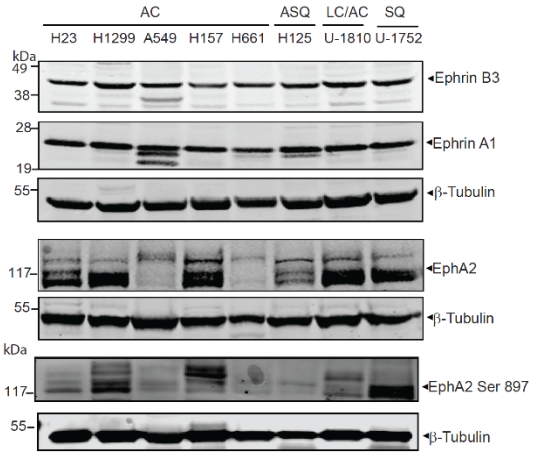
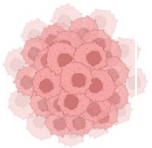
www.impactjournals.com/oncotarget/ Oncotarget, Vol. 7, No. 37

Research Paper

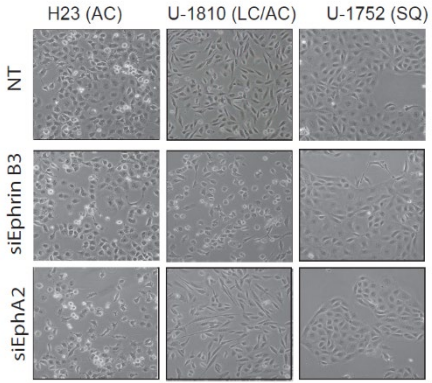
Ephrin B3 interacts with multiple EphA receptors and drives migration and invasion in non-small cell lung cancer

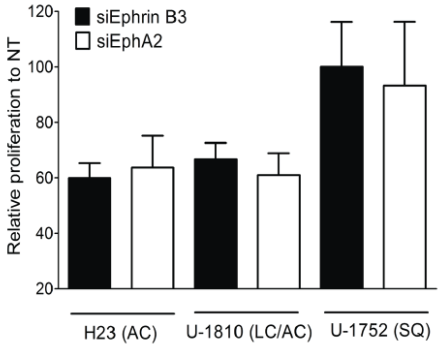
Ghazal Efazat[1,*], Metka Novak[1,*], Vitaliy O. Kaminskyy[2], Luigi De Petris[1], Lena Kanter[1], Therese Juntti[1], Per Bergman[3], Boris Zhivotovsky[2], Rolf Lewensohn[1], Petra Hååg[1], Kristina Viktorsson[1]

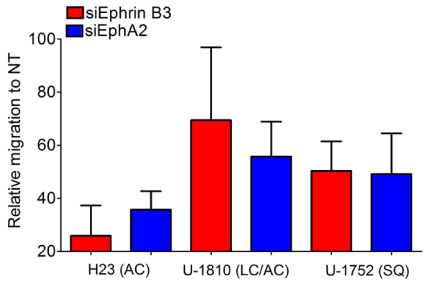




+ Ephrin B3 or EphA2 siRNA







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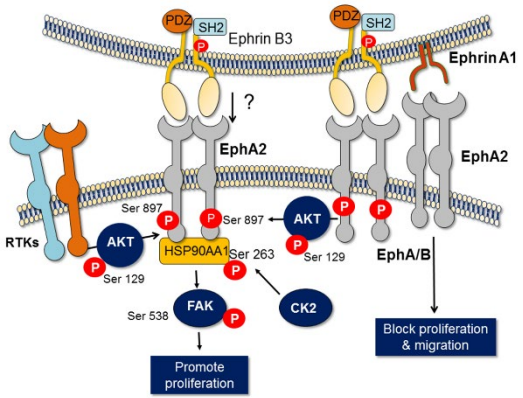

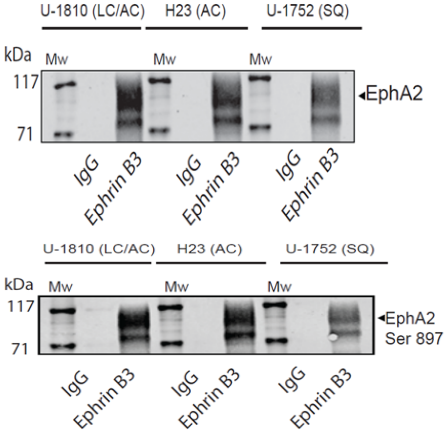

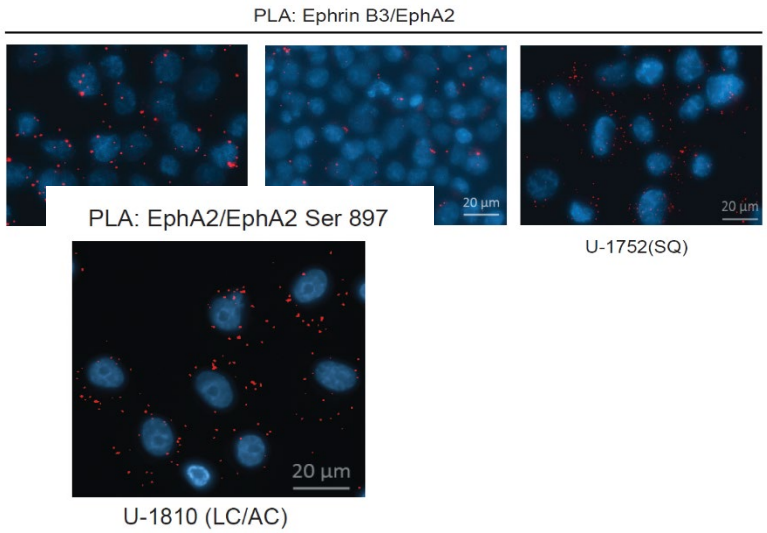

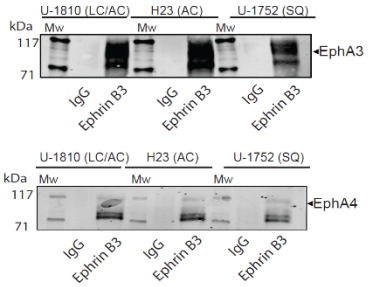

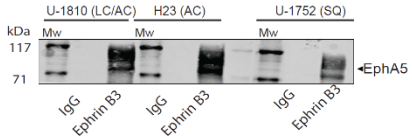

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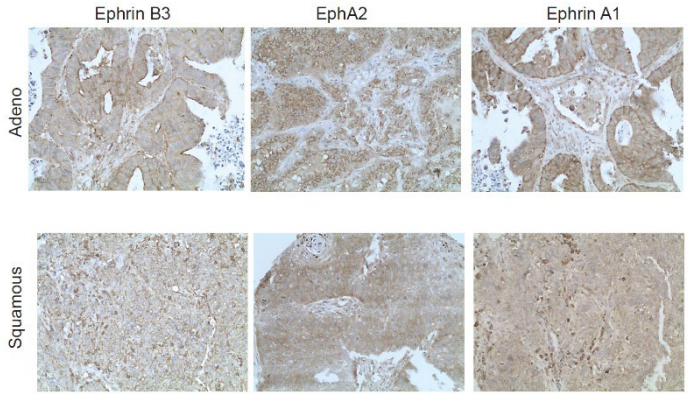
Clinical relevance ?

Brannan JM, Sen B, Saigal B, Prudkin L, Behrens C, Solis L, Dong W, Bekele BN, Wistuba I, Johnson FM. **EphA2 in the early pathogenesis and progression of non-small cell lung cancer**. Cancer Prev Res (Phila). 2009 Dec;2(12):1039-49.

Brannan JM, Dong W, Prudkin L, Behrens C, Lotan R, Bekele BN, Wistuba I, Johnson FM. **Expression of the receptor tyrosine kinase EphA2 is increased in smokers and predicts poor survival in non-small cell lung cancer**. Clin Cancer Res. 2009 Jul 1;15(13):4423-30. doi: 10.1158/1078-0432.CCR-09-0473. Epub 2009 Jun 16. PMID: 19531623.



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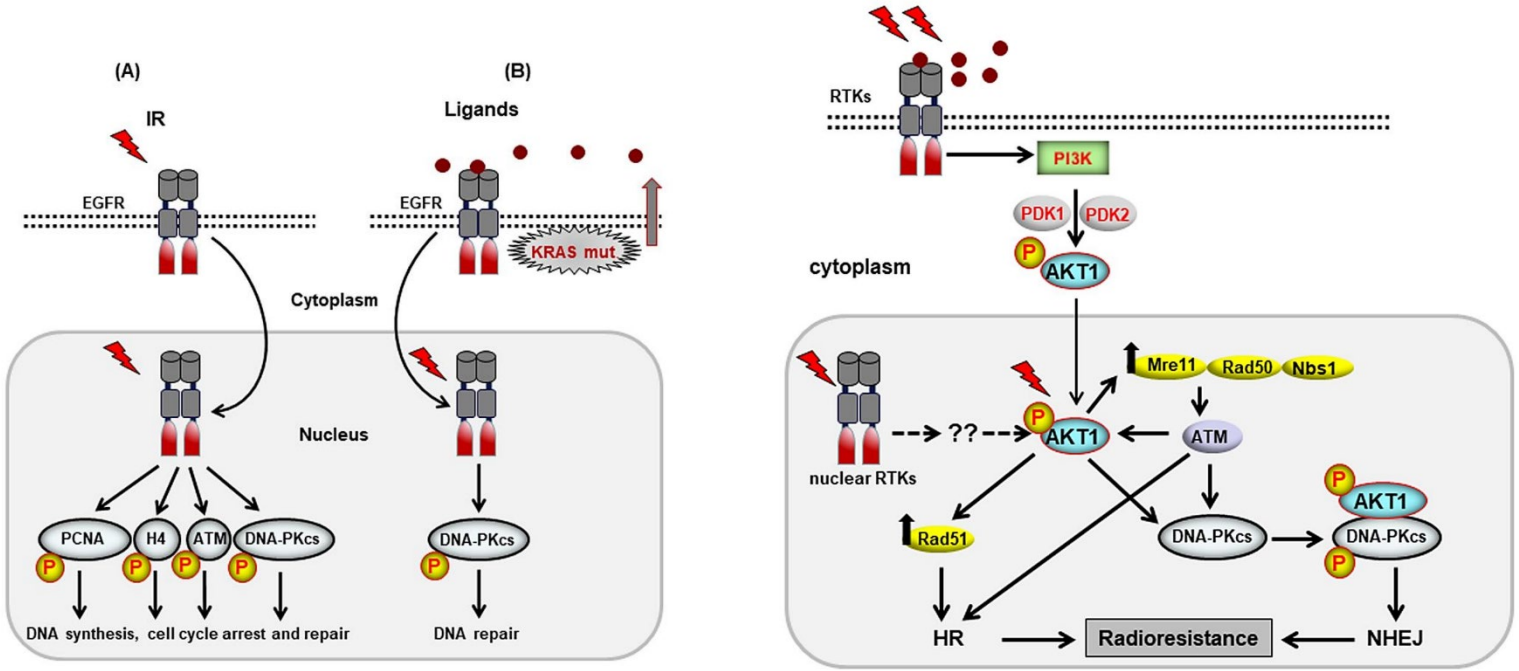
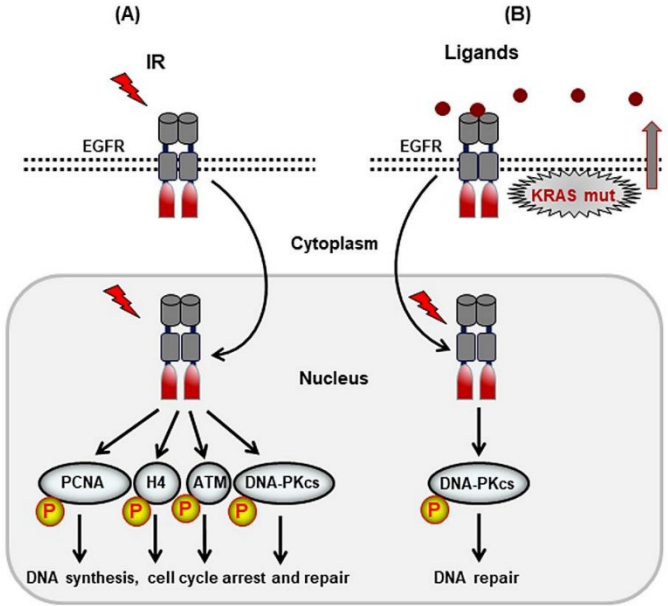
Growth factor receptors can interfere with DNA damage signaling -EGFR as an example



Toulany M. Targeting DNA Double-Strand Break Repair Pathways to Improve Radiotherapy Response. Genes (Basel). 2019 Jan 4;10(1):25. Open acess. Please see guidlines how to use.

Precision cancer medicine agents enhances RT response

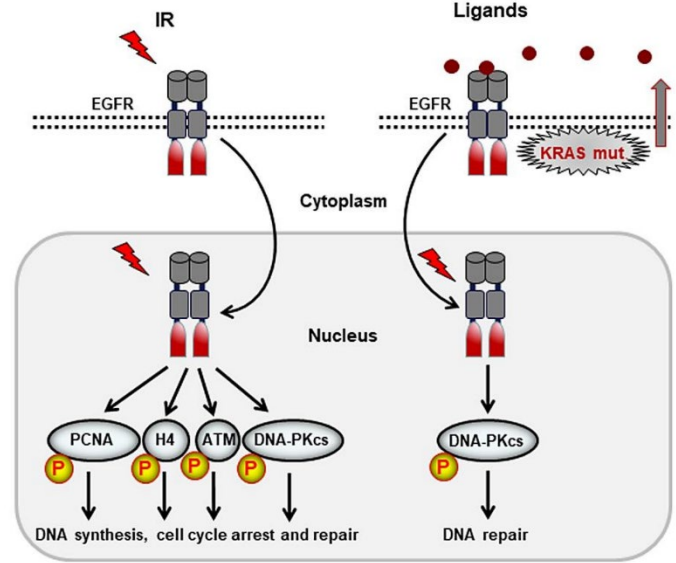
Target/Drug	Combination	Tumor Type	Outcome	Reference
EGFR/Cetuximab	RT	HNSCC	Improved OS	[46,47]
	CRT	NSCLC/Stage III	No improved OS	[53]
	CRT	Esophageal carcinoma	Reduced OS	[54]
	CRT	HNSCC	No improved OS	[55]
	RT vs. CRT	HPV-positive oropharyngeal carcinoma	Lower PFS after cetuximab + RT compared to CRT	[56]
EGFR/Erlotinib	RT	NSCLC	OS 62.5% (3 years)	[57]
	RT	Advanced or metastatic NSCLC	OS 30% (3 years)	[58]
	SBRT	NSCLC	PFS and OS greater than historical values	[59]
	CRT	NSCLC	Effective maintenance therapy PFS 63.5%	[60]
	Bavacizumab + CRT	HNSCC	OS 71% and PFS 82% (3 years)	[61]
	CRT	GM	No improvement in OS and PFS	[62]

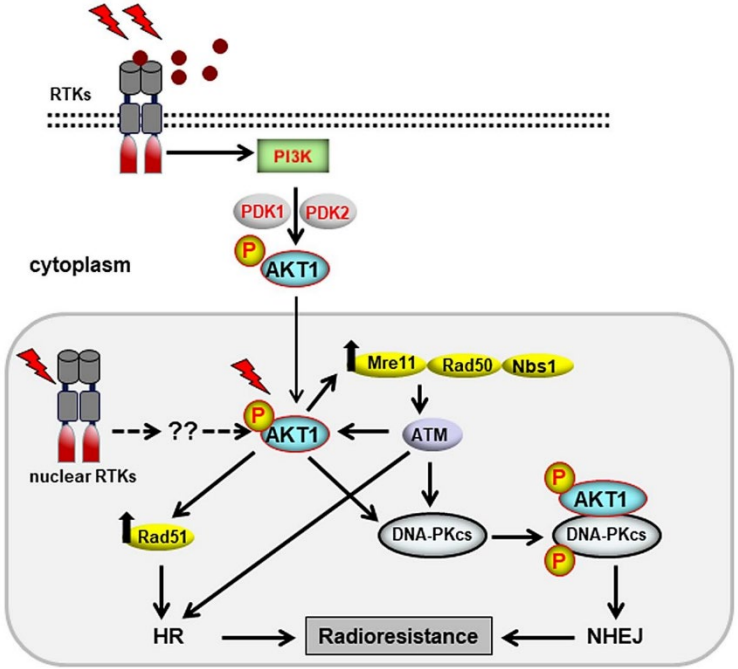


Toulany M. Targeting DNA Double-Strand Break Repair Pathways to Improve Radiotherapy Response. Genes (Basel). 2019 Jan 4;10(1):25. Open acess. Please see guidlines how to use.

Growth factor receptors can interfere with DNA damage signaling -EGFR as an example

What about EphA:s?



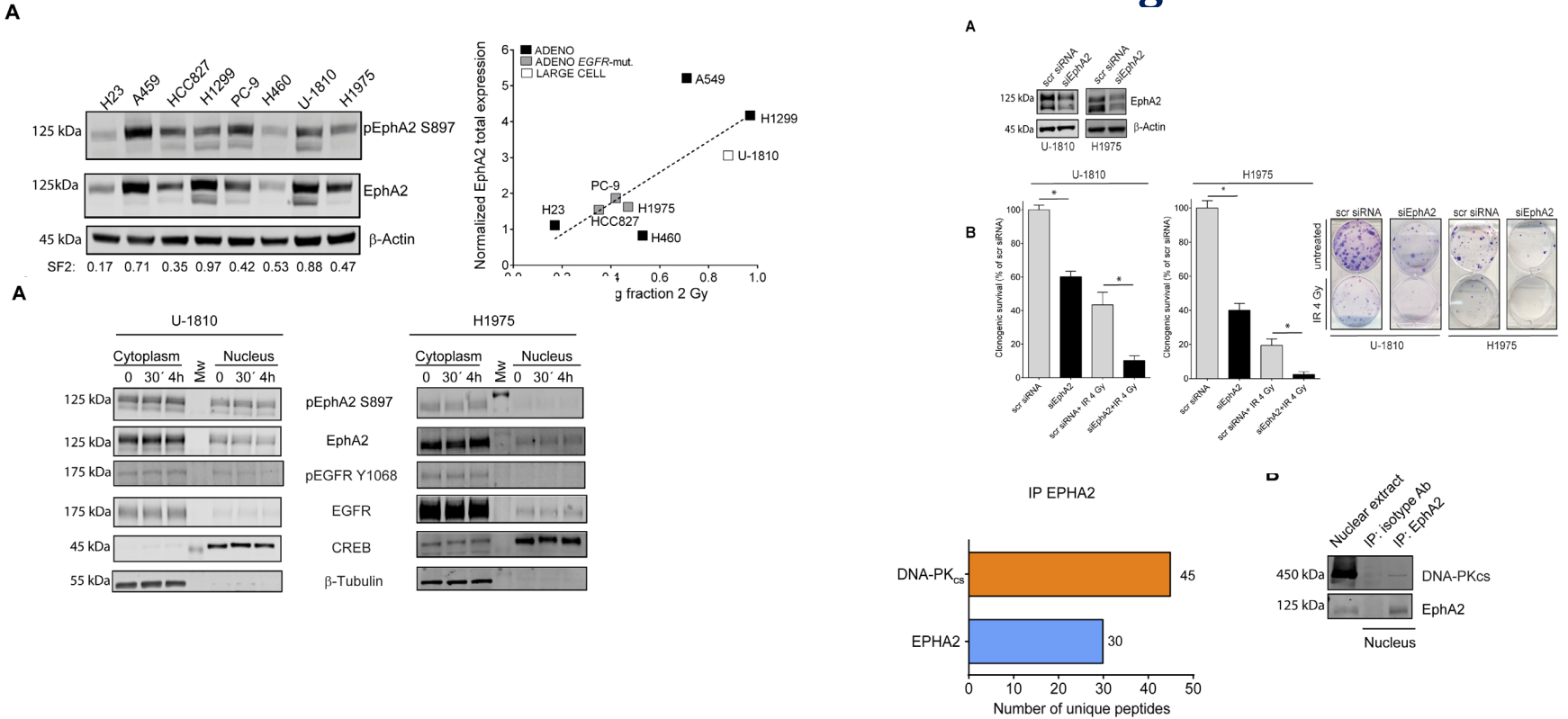


Toulany M. Targeting DNA Double-Strand Break Repair Pathways to Improve Radiotherapy Response. Genes (Basel). 2019 Jan 4;10(1):25. Open acess. Please see guidlines how to use.

EphA5 control RT response in NSCLC cells and interact with ATM

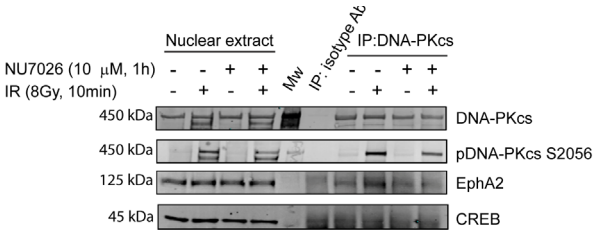
Reference: Staquicini FI, Qian MD, Salameh A, Dobroff AS, Edwards JK, Cimino DF, Moeller BJ, Kelly P, Nunez MI, Tang X, Liu DD, Lee JJ, Hong WK, Ferrara F, Bradbury AR, Lobb RR, Edelman MJ, Sidman RL, Wistuba II, Arap W, Pasqualini R. Receptor tyrosine kinase EphA5 is a functional molecular target in human lung cancer. J Biol Chem. 2015 Mar 20;290(12):7345-59.

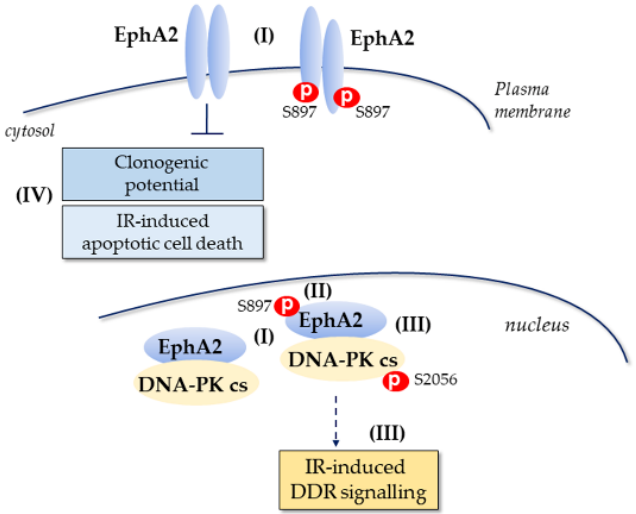
EPHA2 Interacts with DNA-PK $_{cs}$ in Cell Nucleus and Controls Ionizing Radiation Responses in Non-Small Cell Lung Cancer Cells



Kaminskyy, V. O., P. Hååg, M. Novak, Á. Végvári, V. Arapi, R. Lewensohn and K. Viktorsson (2021). "EPHA2 Interacts with DNA-PKcs in Cell Nucleus and Controls Ionizing Radiation Responses in Non-Small Cell Lung Cancer Cells." Cancers **13**(5): 1010.

EPHA2 Interacts with DNA-PK $_{cs}$ in Cell Nucleus and Controls Ionizing Radiation Responses in Non-Small Cell Lung Cancer Cells





Kaminskyy, V. O., P. Hååg, M. Novak, Á. Végvári, V. Arapi, R. Lewensohn and K. Viktorsson (2021). "EPHA2 Interacts with DNA-PKcs in Cell Nucleus and Controls Ionizing Radiation Responses in Non-Small Cell Lung Cancer Cells." Cancers **13**(5): 1010.

Conclusions

- There are ongoing important development targeting DNA-PKcs in clinical trials.
- For tumor lacking functional BRCA1 attempts are made with PARP inhibitors also in combination with RT.
- High LET IR triggers a different signaling cascade in NSCLC cells than low LET which involves early and late JNK activation followed by mitochondria mediated apoptosis.
- Heterogenity within tumor cells and tumors influence RT response as exemplified with tumor initiating cells.
- Ephrin B3, a ligand of multiple EphAs controls multiple functions in NSCLC cells including RT sensitivity, cell cycle and cell death signaling.
- EphA2 is a growth factor receptor driving multiple functions in tumor cells including migration, invasion and cellular response to IR.
- EGFR and EphA5 both influence RT response in multiple ways including effect on DNA damaging signaling cascades.
- EphA2 interacts with the NHEJ component DNA-PKcs in cell nucleus.
- RT biomarkers and targets are in tumors also influenced by immune cell signaling and tissue heterogenity.

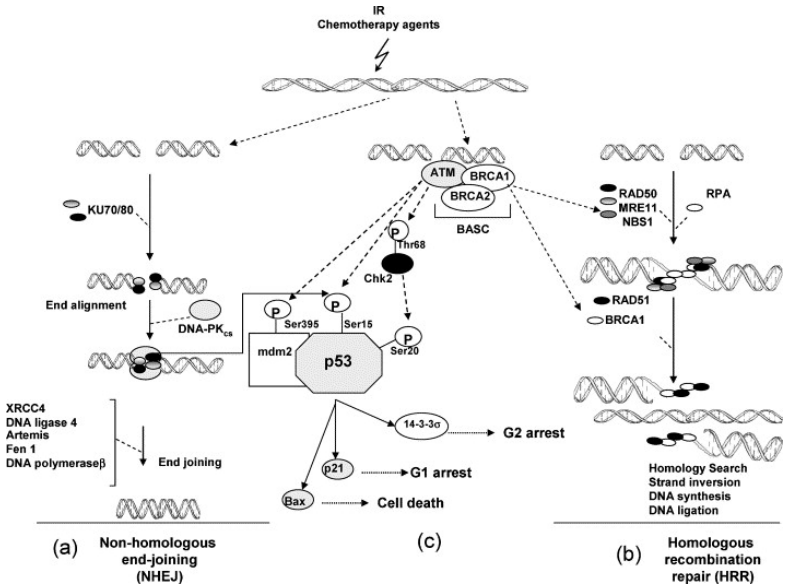
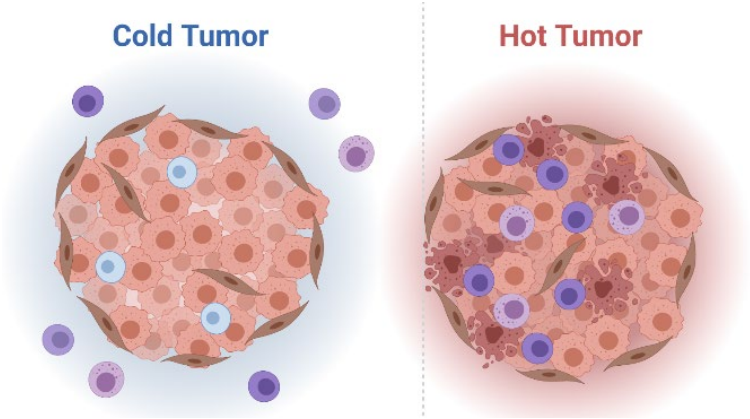
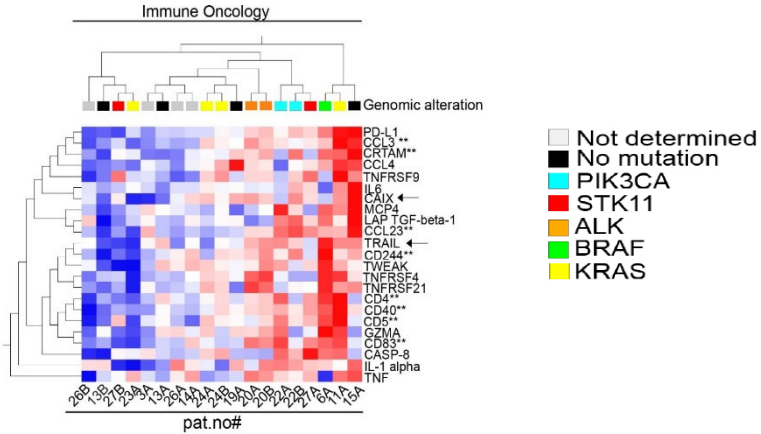


Fig.1
Viktorsson K, De Petris L, Lewensohn R. The role of p53 in treatment responses of lung cancer. Biochem Biophys Res Commun. 2005 Jun 10;331(3):868-80.

Beyond molecular & genetic biomarkers? Whats next?





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Beyond molecular & genetic biomarkers? Whats next? Exosomes important communicators

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