Molecular tumour response to accelerated ions –and role of cellular modifying factors - swedish research

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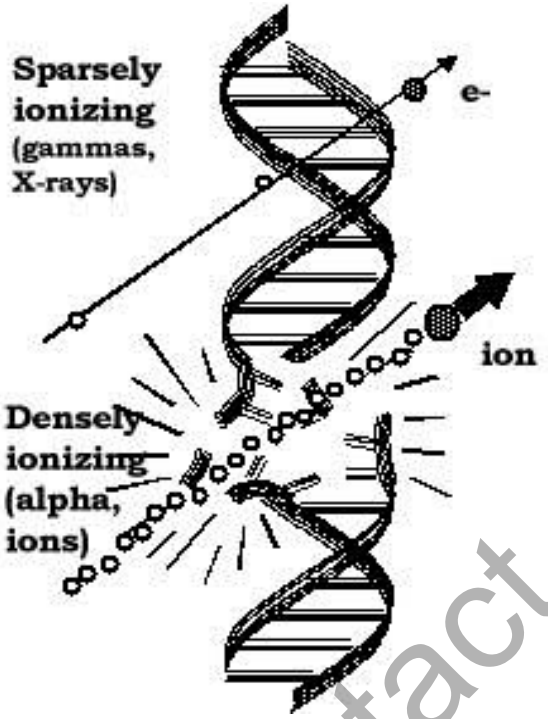
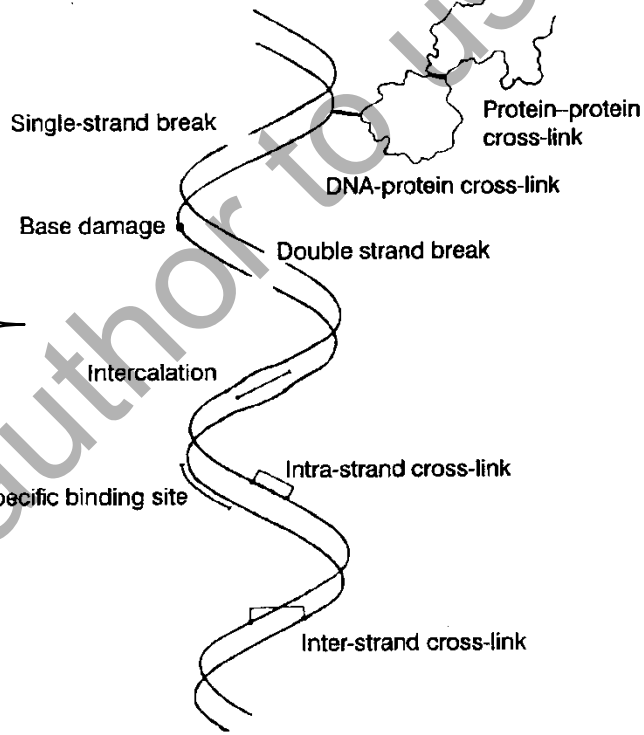
ENLIGHT meeting, Krakow
18th of September 2015

Accelerated particles & complex DNA damages - biological consequences ?

Low LET IR

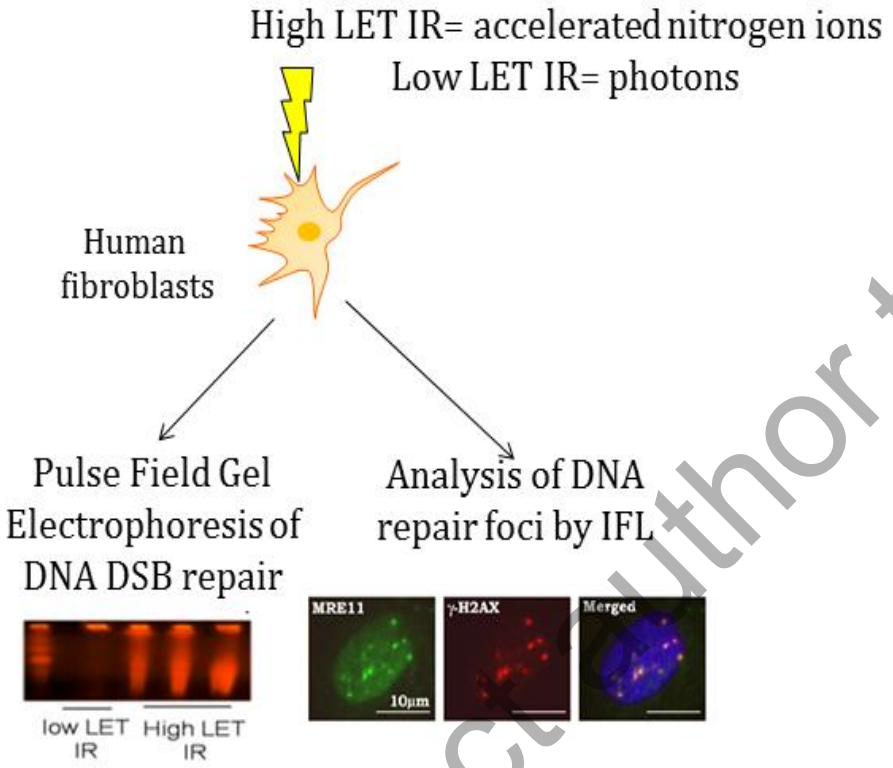


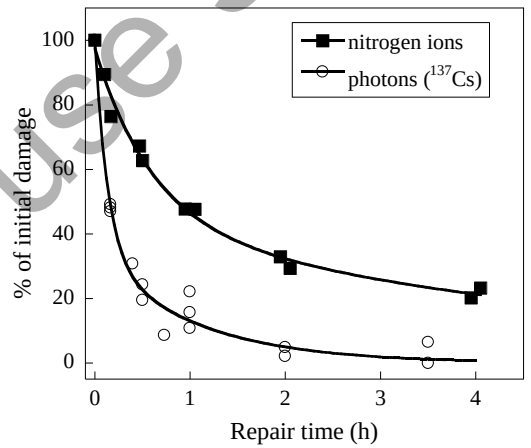
High LET IR



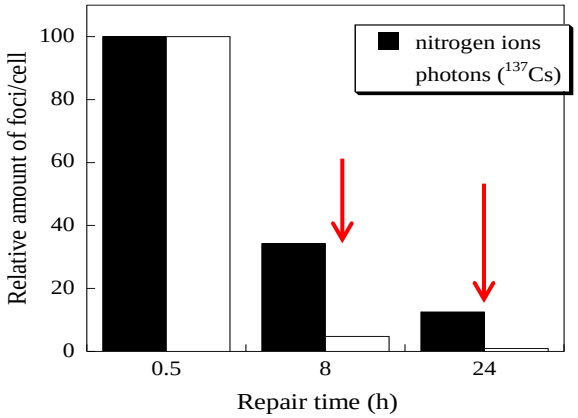
Different signaling cascades

Accelerated particles induces complex less repairable DNA damages



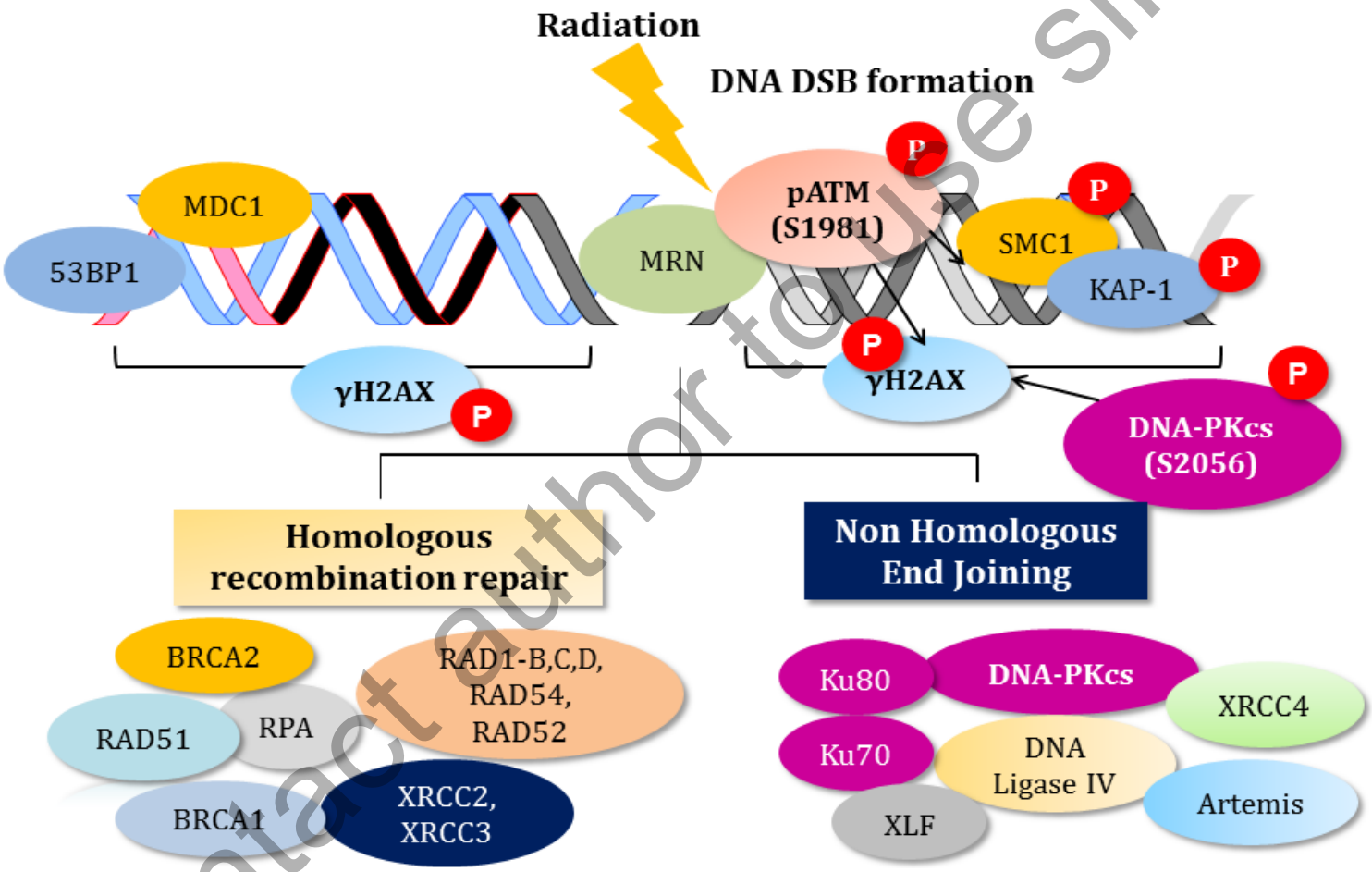


γH2AX foci

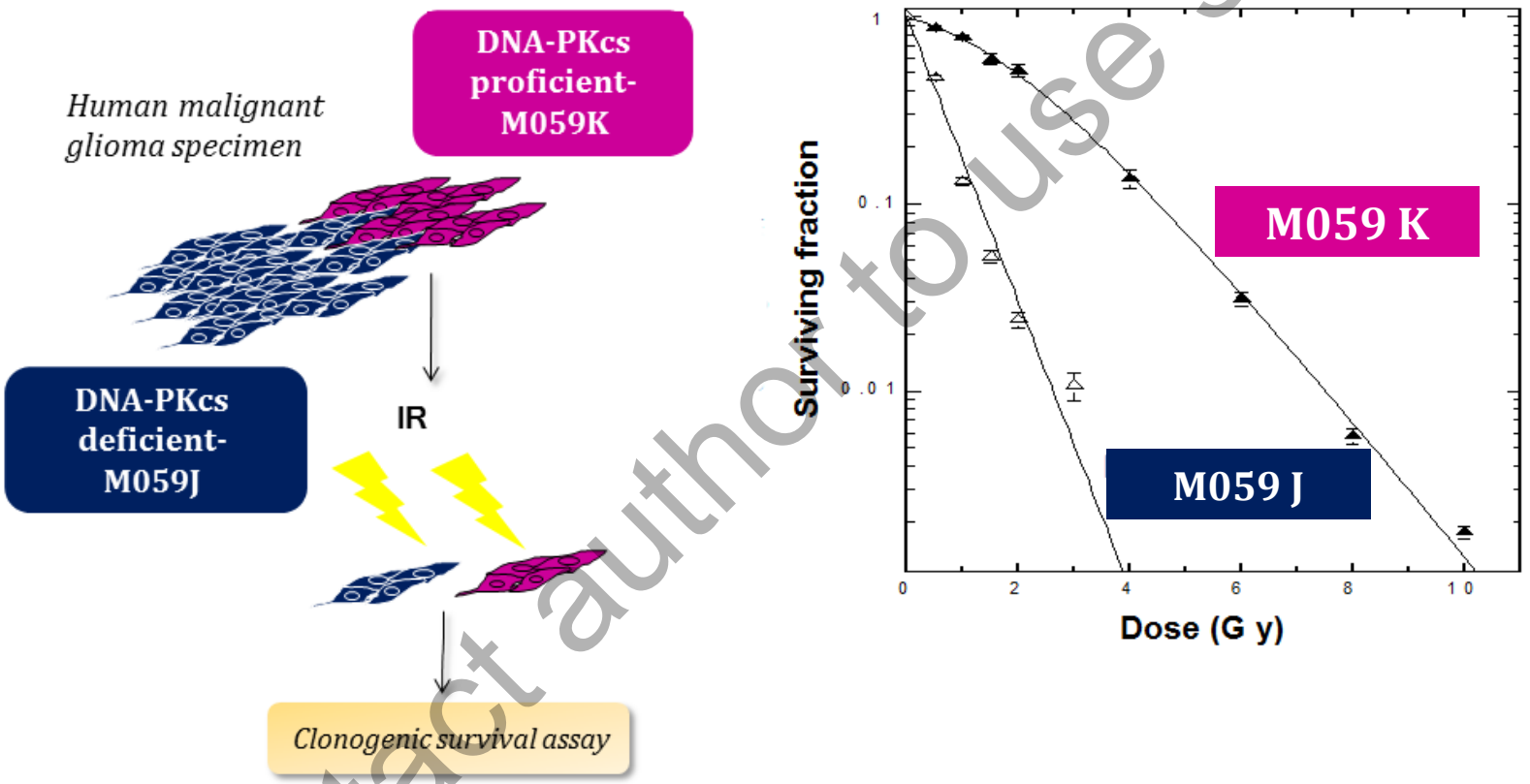


Data from collaborator Prof. Stenerlöw, Uppsala University, Sweden.

Cellular DNA damage response & repair pathways

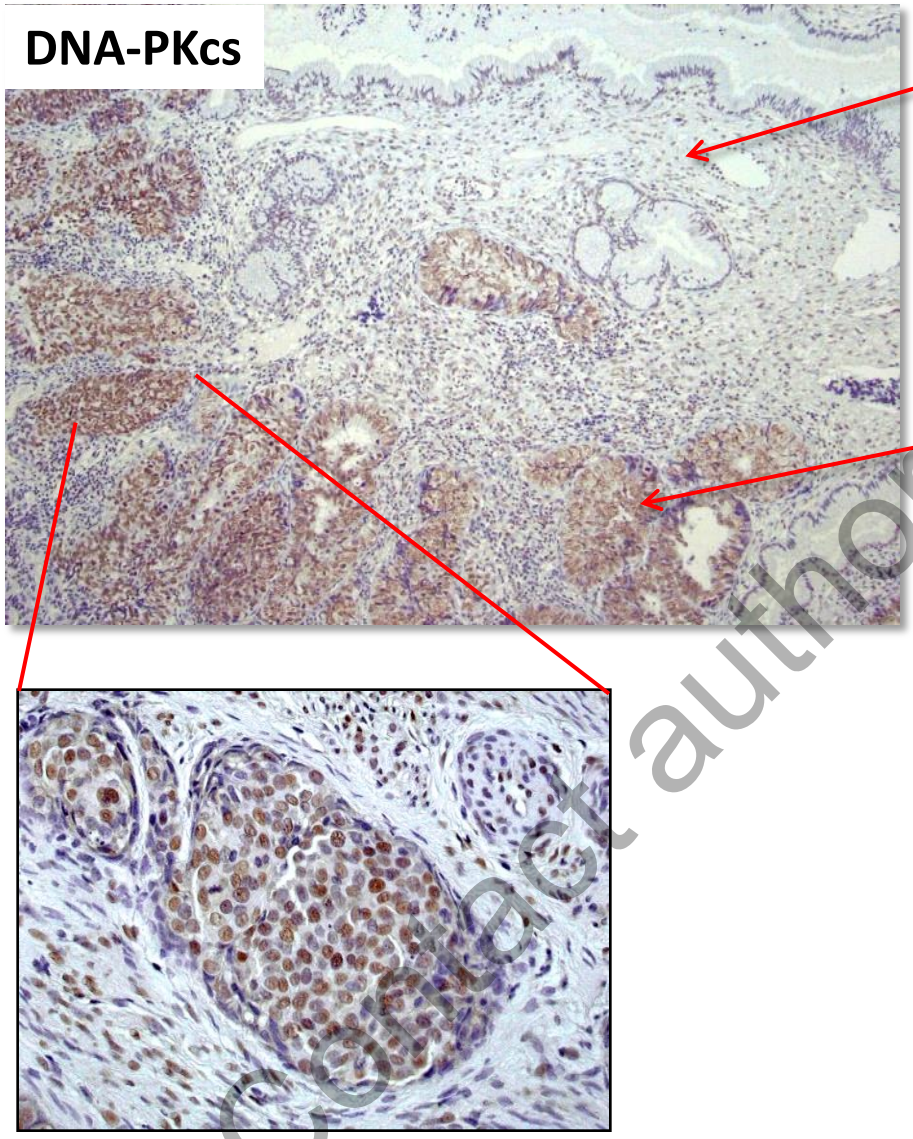


A role of DNA-PKcs in low LET IR response



References: Allalunis-Turner, M. J., et al. (1993). *Radiat Res* **134**(3): 349-354. Lind, B. K., et al. (2003). *Radiat Res* **160**(3): 366-375.

Increased DNA-PK signaling confers RT resistance *in vivo*



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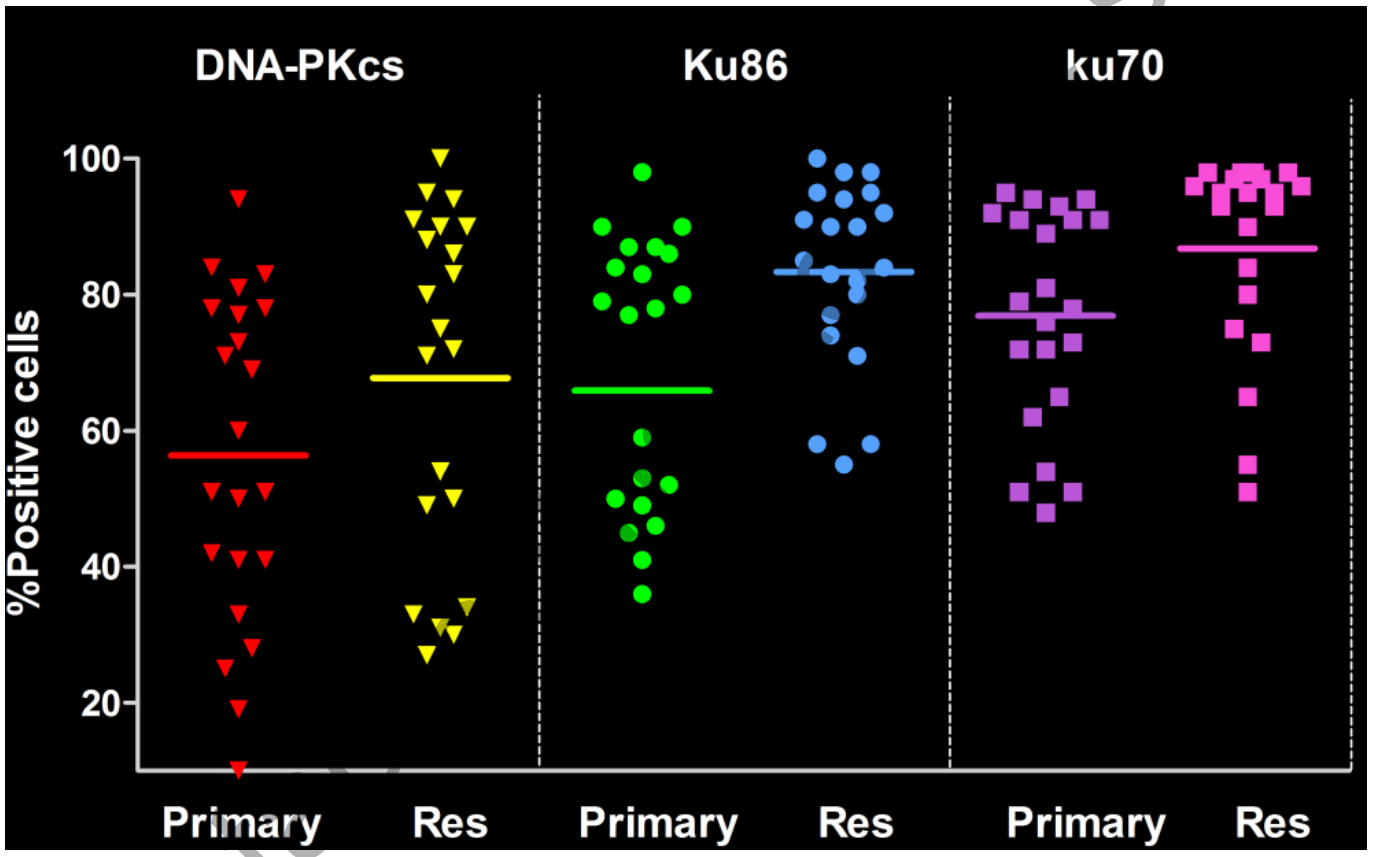
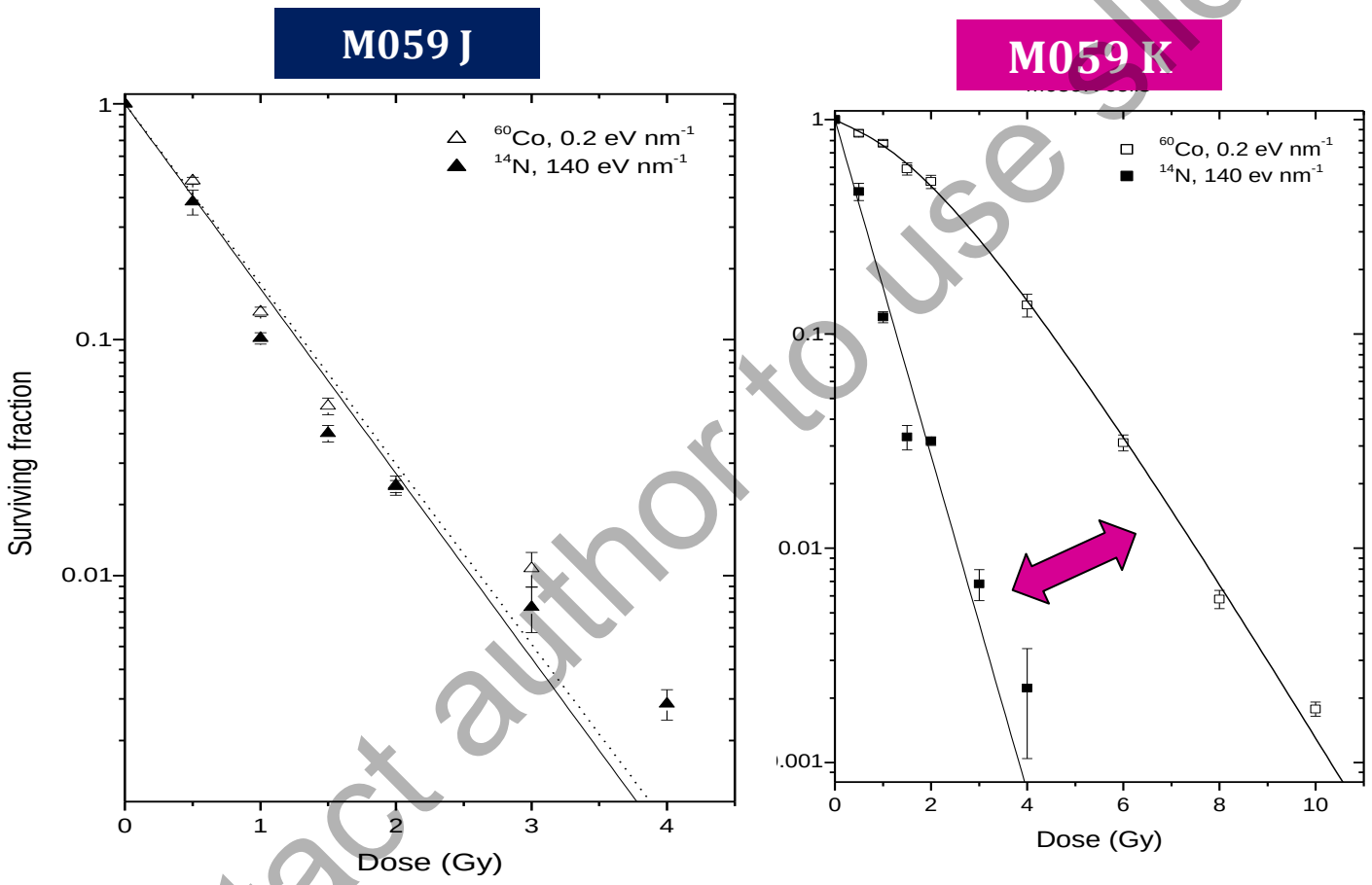


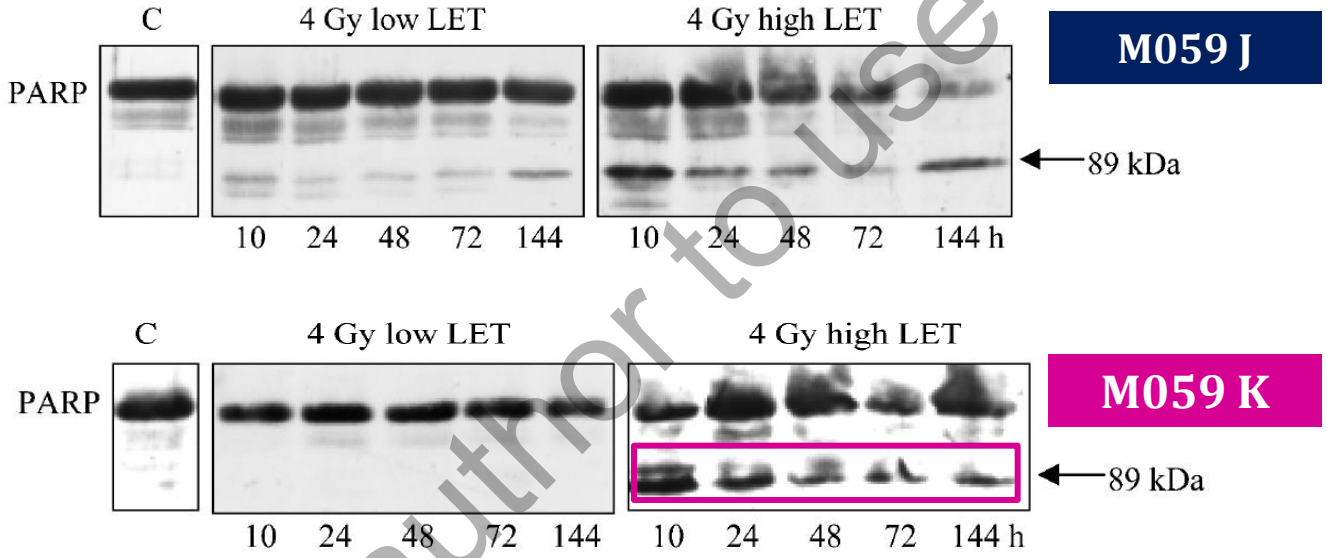
Reference: Beskow, C., et al. (2009). *Br J Cancer* **101**(5): 816-821.

High LET IR can overcome DNA-repair mediated by DNA-PKcs



Holgersson A, Jernberg AR, Persson LM, Edgren MR, Lewensohn R, Nilsson A, Brahme A, Meijer AE. Low and high LET radiation-induced apoptosis in M059J and M059K cells. *Int J Radiat Biol*. 2003 79:611-21.

High LET IR can overcome DNA-repair mediated by DNA-PKcs and trigger apoptotic signaling



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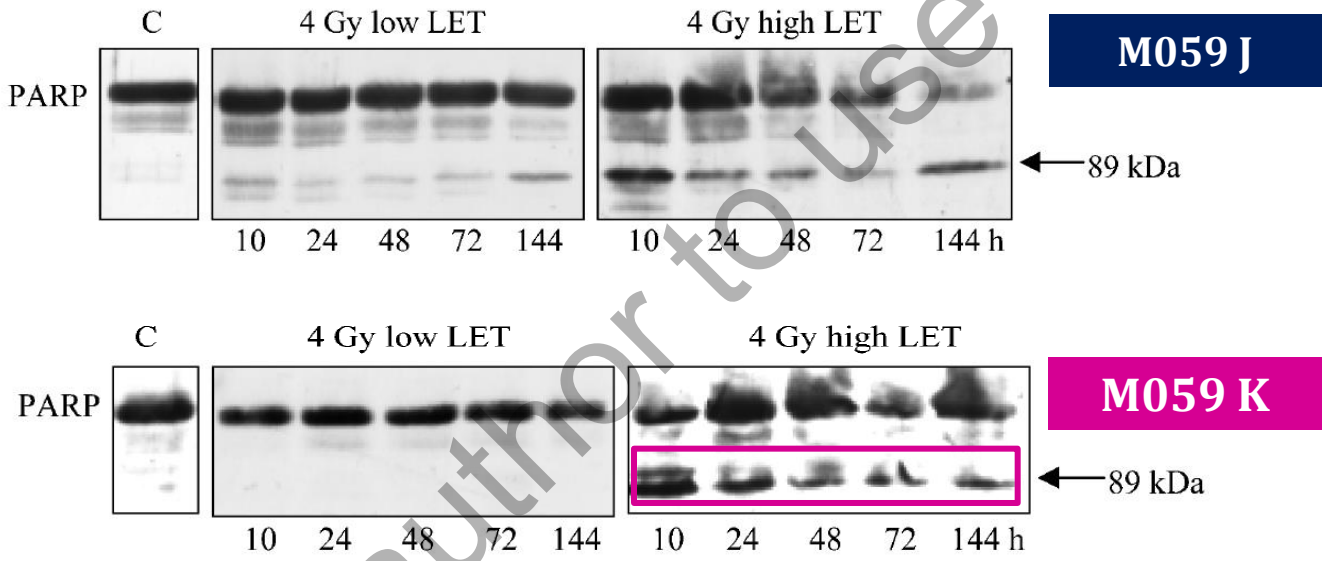
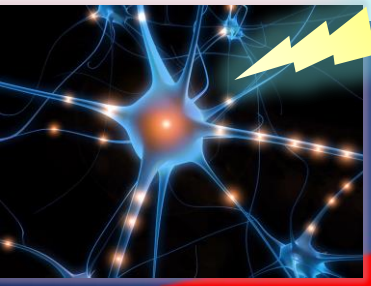


- High LET IR can circumvent functional DNA-PKcs, ATM or p53 to trigger cell death.

Holgersson A, Jernberg AR, Persson LM, Edgren MR, Lewensohn R, Nilsson A, Brahme A, Meijer AE. Low and high LET radiation-induced apoptosis in M059J and M059K cells. *Int J Radiat Biol*. 2003 79:611-21.

High LET IR can bypass different DNA repair events- what about other signaling cascades ?



Activation of growth factor receptors

EGFR, IGFR, PDGF

DNA damage surveillance

cell survival signalling

p53

ATM

Cell cycle regulation

p21

Chk1/Chk2

cell death signallning

Activation of DNA-repair

Ku70 DNA-PKcs

Ku80

DNA DSBs:
NHEJ, HR
Base damages:
BER
Crosslinks: MMR

Apoptosis
Mitotic catastrophe
Necrosis

Correct DNA repair

Incorrect DNA repair

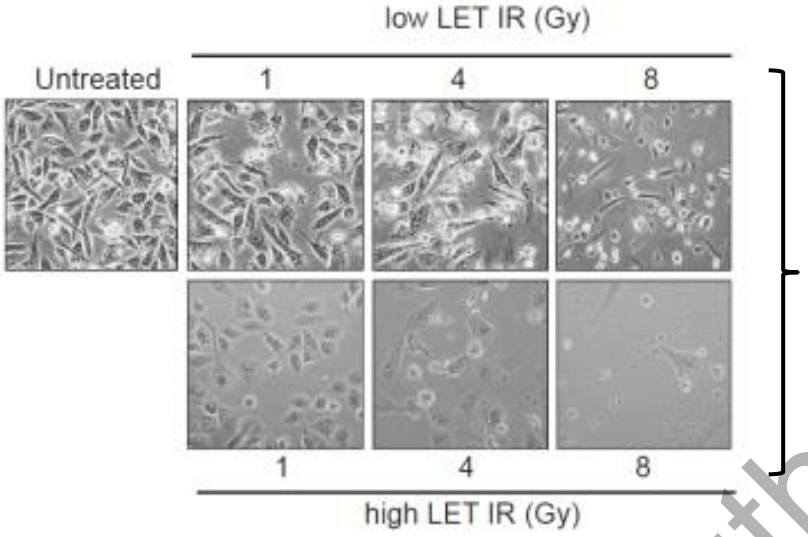
Cell survival

Cell with mutation

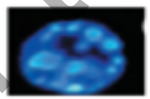
Cell death

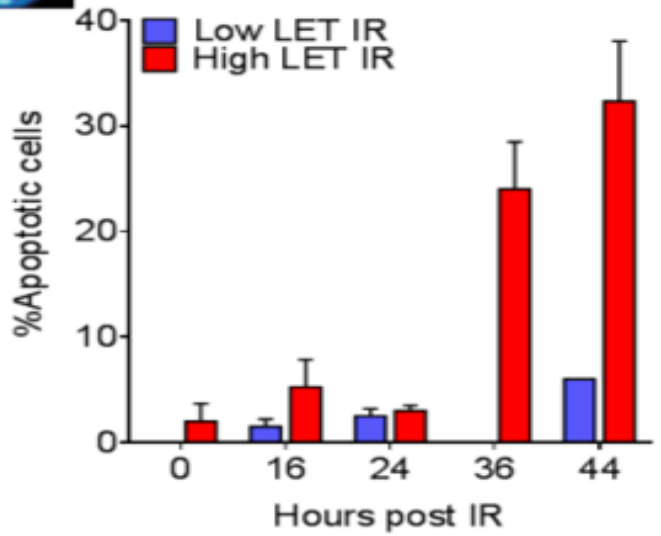
High LET IR can overcome low LET IR resistance and trigger apoptotic signalling

Non small cell lung cancer cells



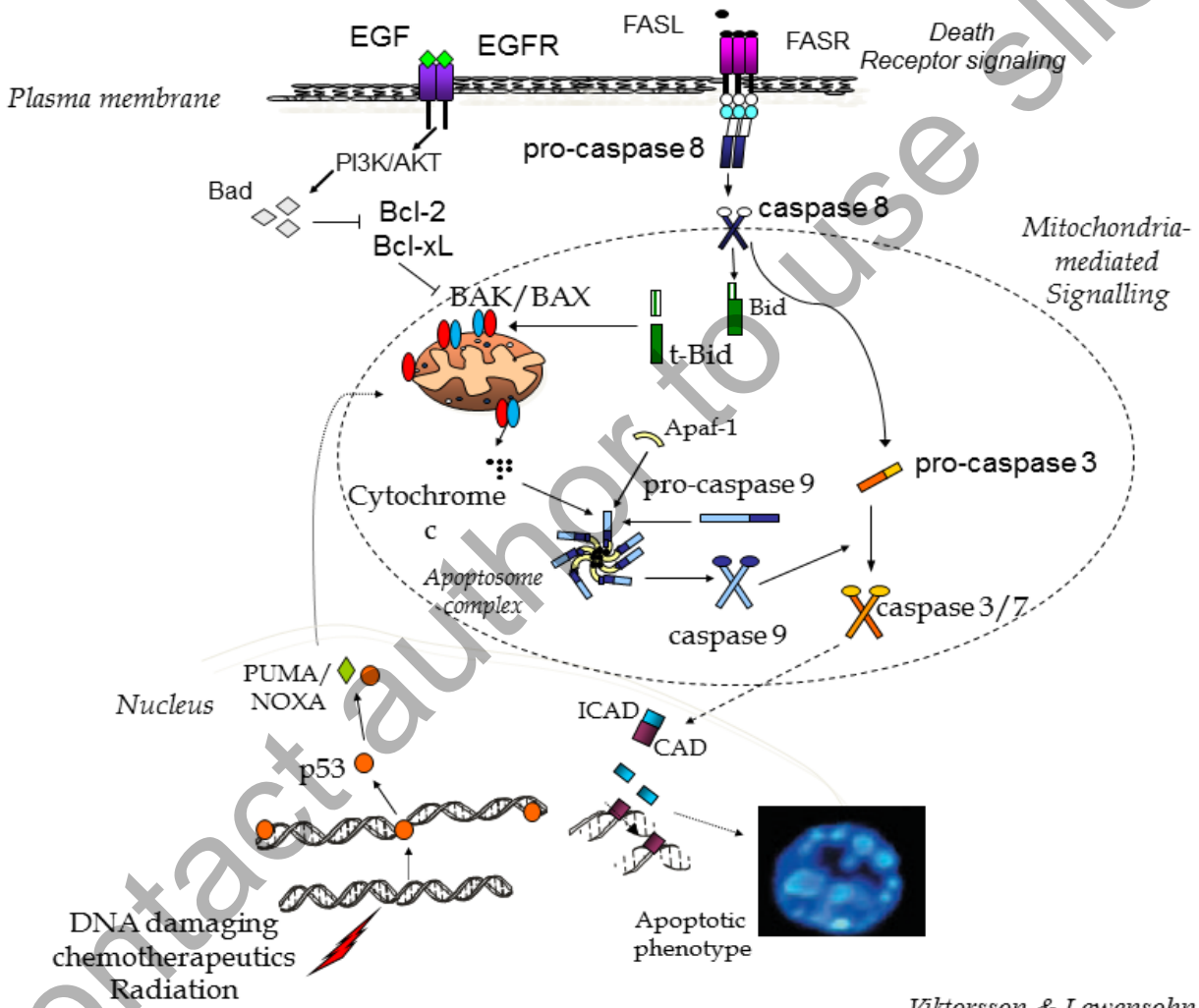
What are the apoptotic signaling events triggered after high but not low LET IR ?





Viktorsson et al., Exp. Cell Research, 2003; Stahl et l MCP, 2009

DNA-damage induced apoptotic signaling



Viktorsson & Lewensohn, JTO, 2007

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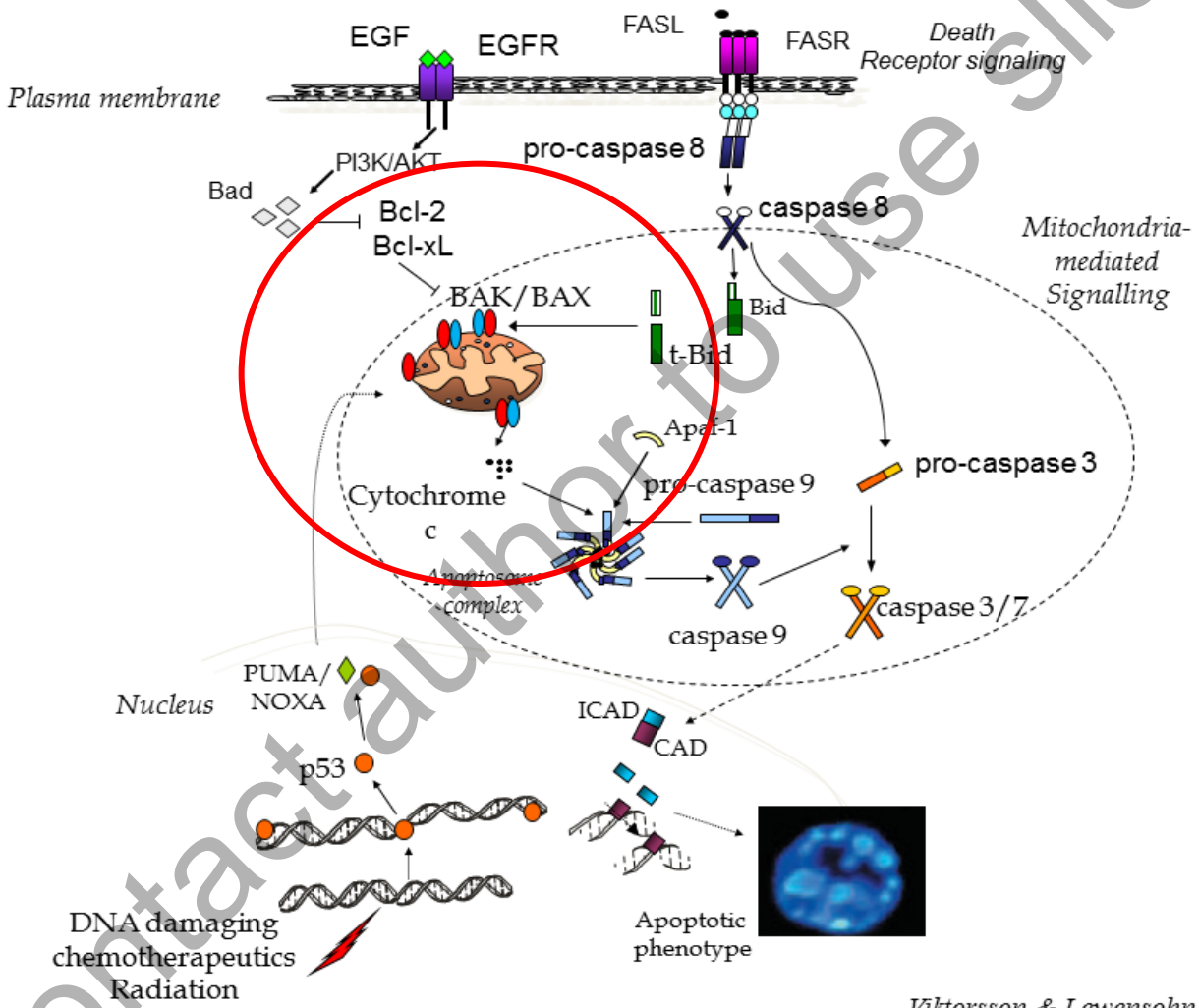
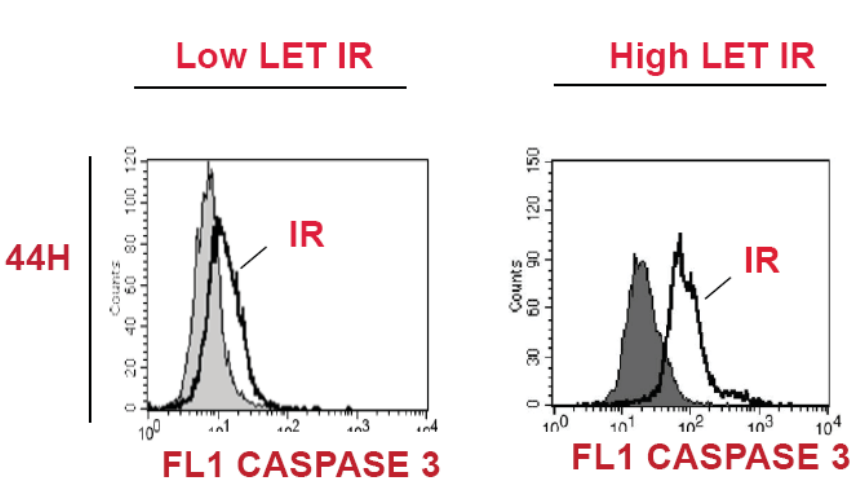
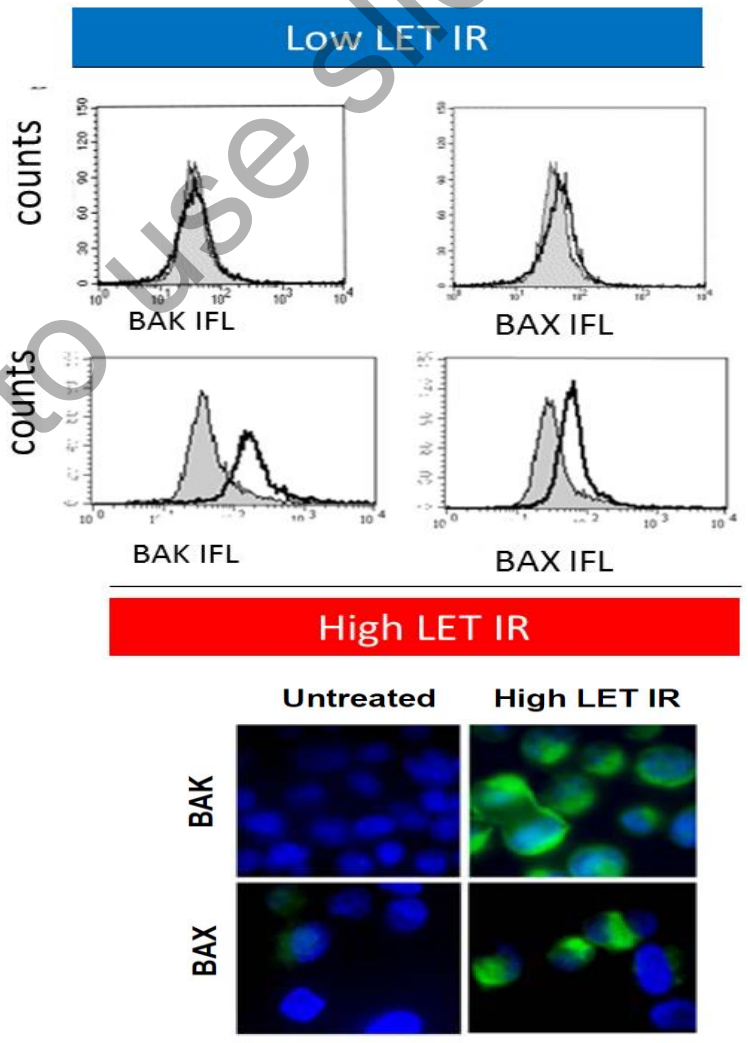


Viktorsson & Lewensohn, JTO, 2007

High LET IR activates mitochondria-mediated signalling





Viktorsson et al., Exp. Cell Research, 2003; Stahl et l MCP, 2009

Proteomic-based characterization of high LET IR response in tumor cells

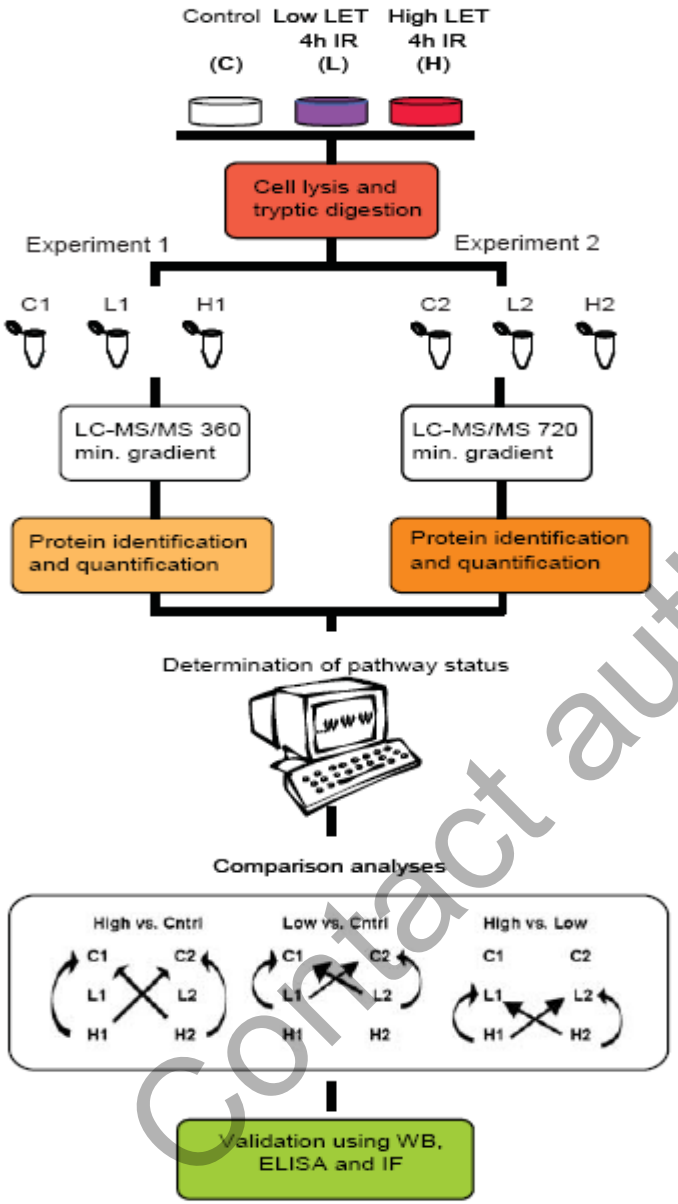
Molecular & Cellular PROTEOMICS

Submitted on June 18, 2008
Revised on January 21, 2009
Accepted on January 23, 2009

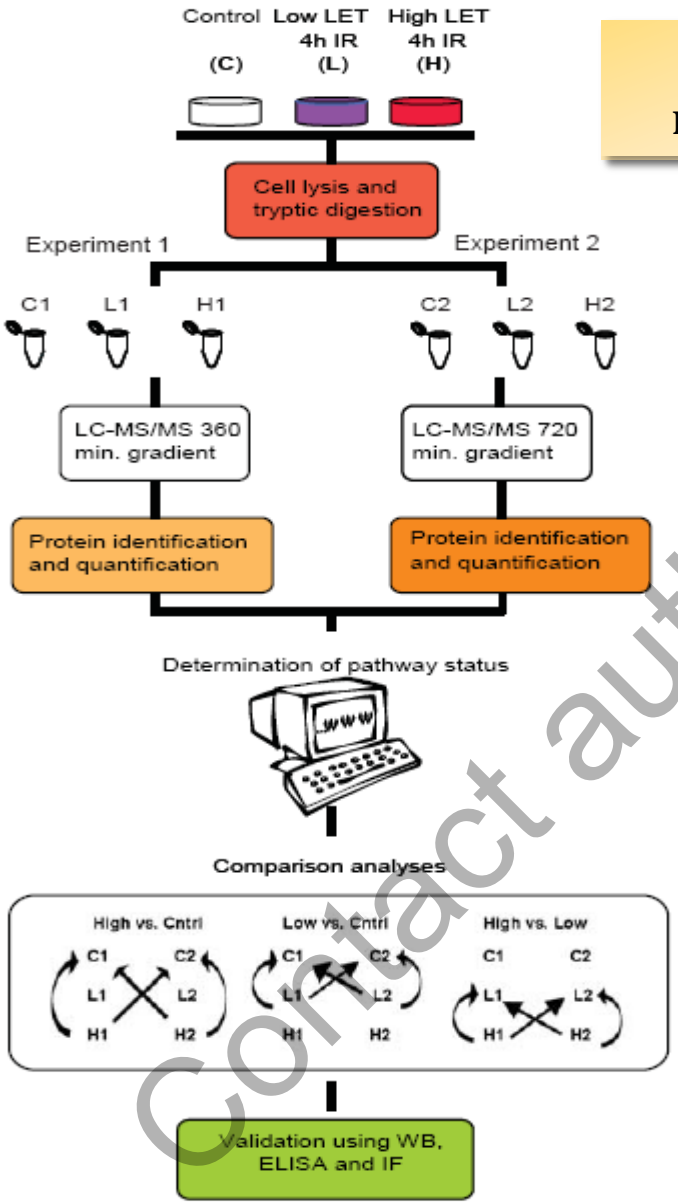
Proteomics and pathway analysis identifies JNK-signaling as critical for High-LET radiation-induced apoptosis in non-small lung cancer cells

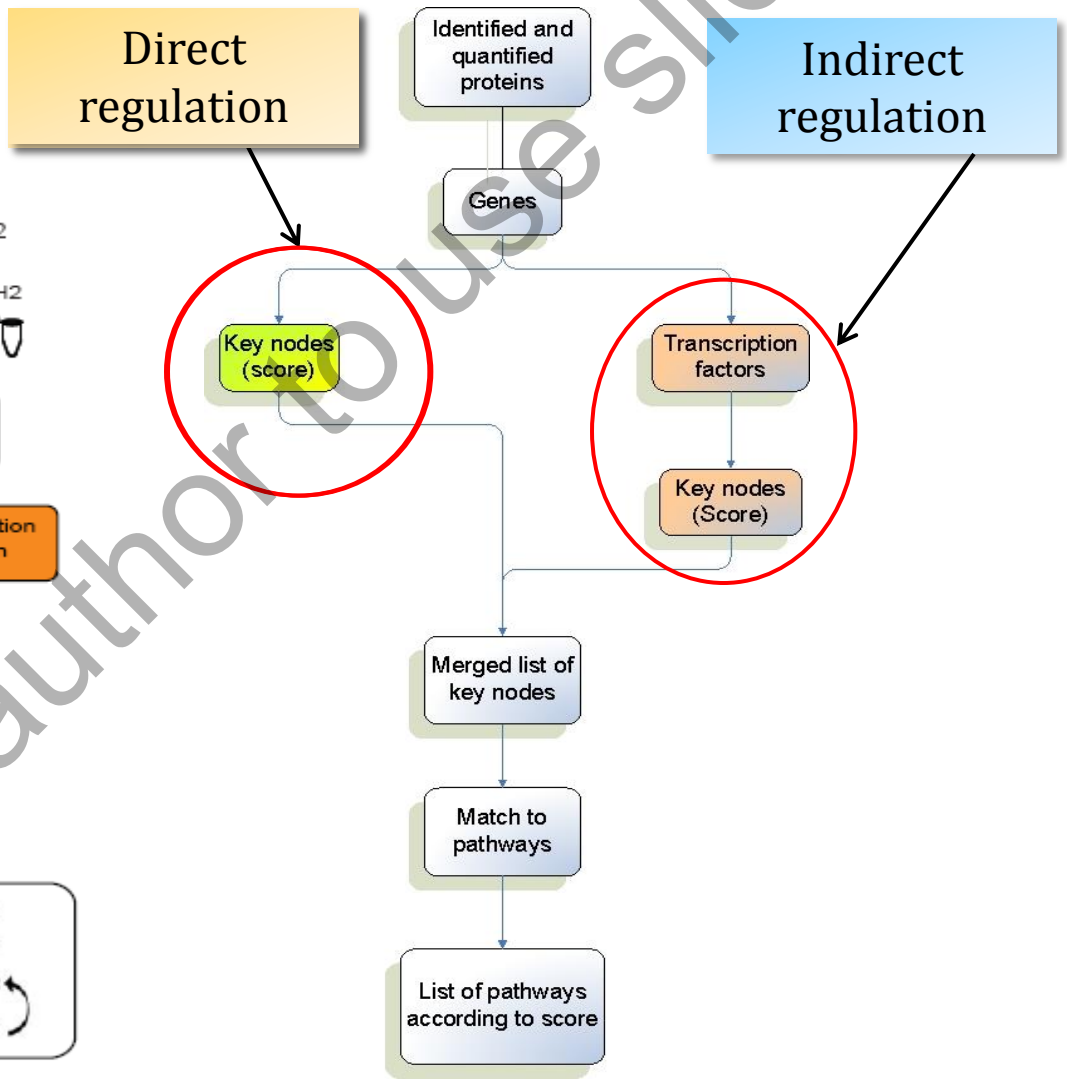
Sara V. Stahl, Eva Fung, Christopher Adams, Johan Lengqvist, Birgitta Mörk, Bo Stenerlöw, Rolf Lewensohn, Janne Lehtiö, Roman Zubarev, and Kristina Viktorsson

Proteomic-based characterization of high LET IR response in tumor cells



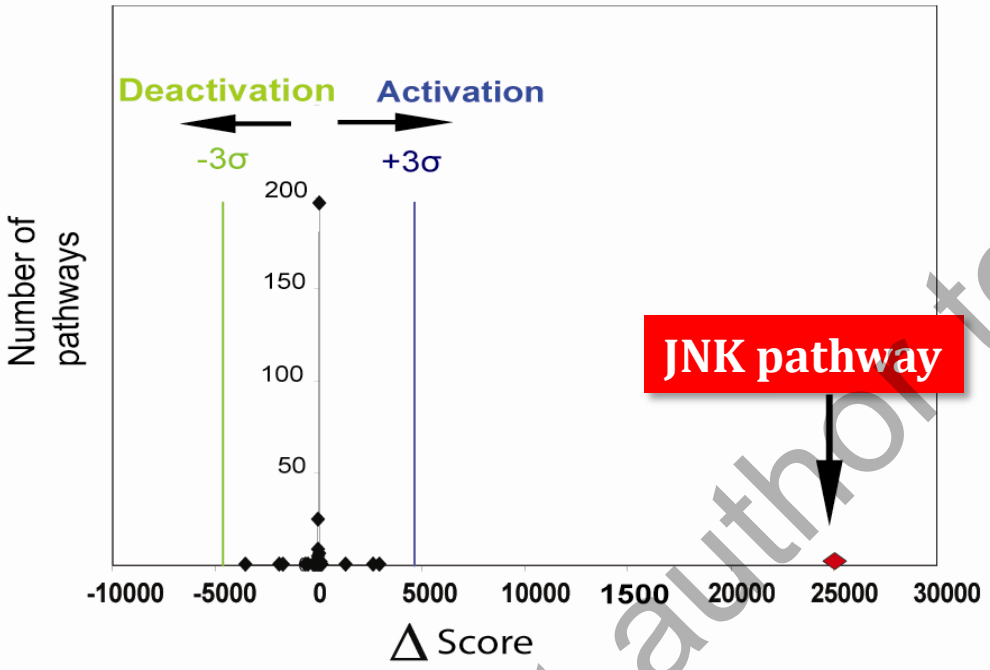
Proteomic-based characterization of high LET IR response in tumor cells





Zubarev et al. 2008, Journal of proteomics

Total proteomic analysis identifies JNK as a critical signalling event after high LET IR



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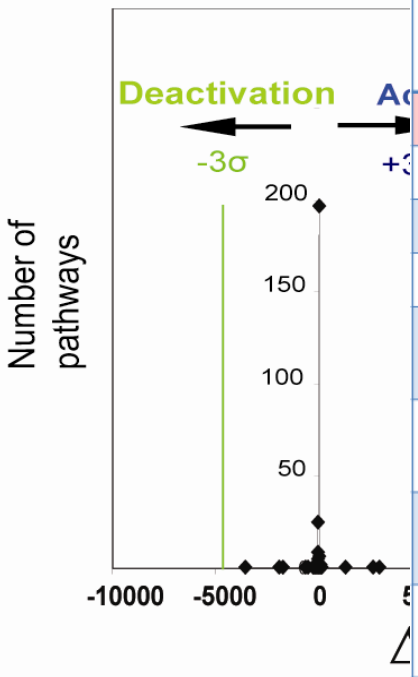


Activated pathways	ΔS	Deactivated Pathways	ΔS
JNK	24897	P38 MAPK	-3561
Fas	2929	RANKL	-1897
EGFR	2625	Epo	-1713
JNK/MKK4	1302	PRL	-633
IL-8	175	BMP2 to p38 MAPK alpha	-519
Neurotensin	151	TAK1 to ATF2	-287
Caspase network	127	EGF-PAK1	-185
LAT to PKC beta	86	Apo2L	-159
		RANK to MITF	-130

Selection of JNK and p38 MAPK pathways for validation

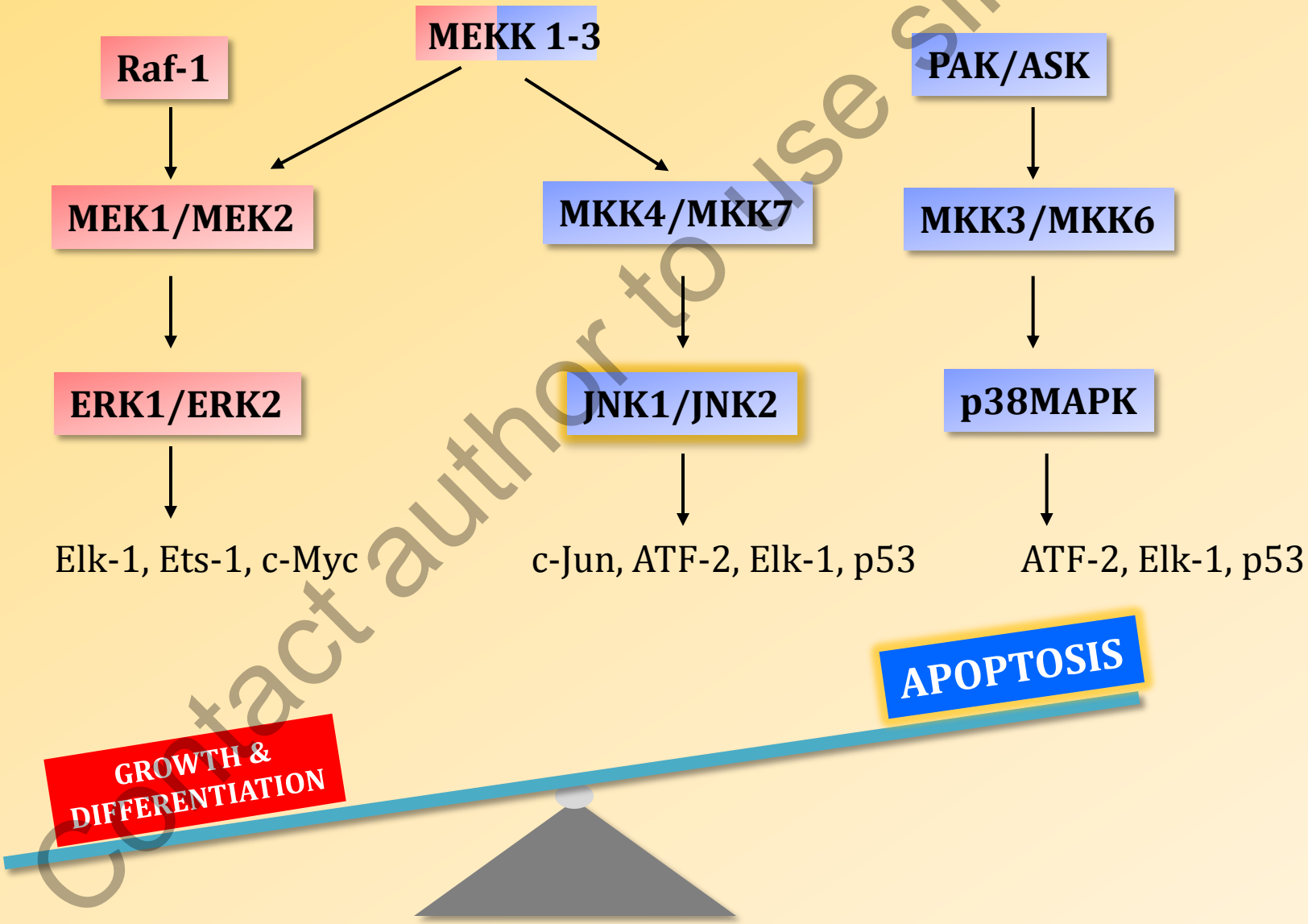
In silico studies suggested the **JNK-pathway** as **activated** and the **p38-pathway** as **deactivated** in response to high LET IR in NSCLC cells

Defective JNK activation in response to low LET IR in NSCLC cells
(Viktorsson et al Exp Cell Res.2003)

p38 activates IGF-1R in response to low-LET IR in NSCLC cells
(Cosaceanu et al , Oncogene. 2007)

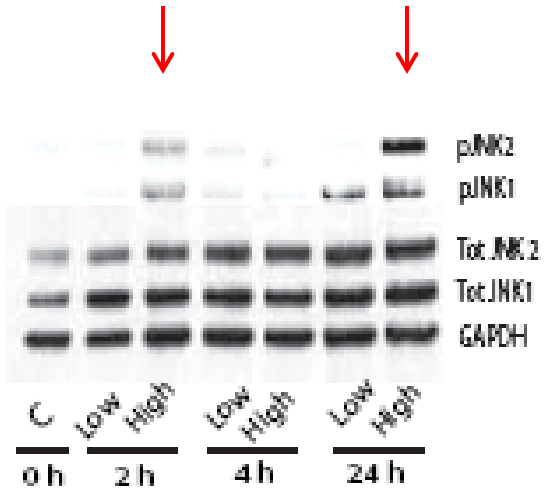
JNK & p38 MAPK

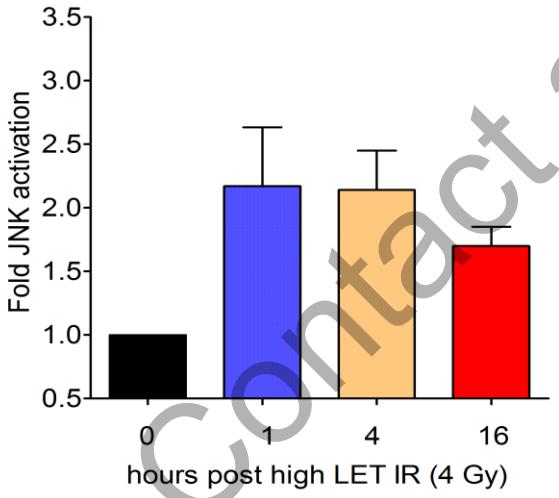
MAPK signalling cascades – regulators of proliferation & cell death



High LET but not low LET IR causes sustained JNK activation Which controls high LET-induced apoptosis

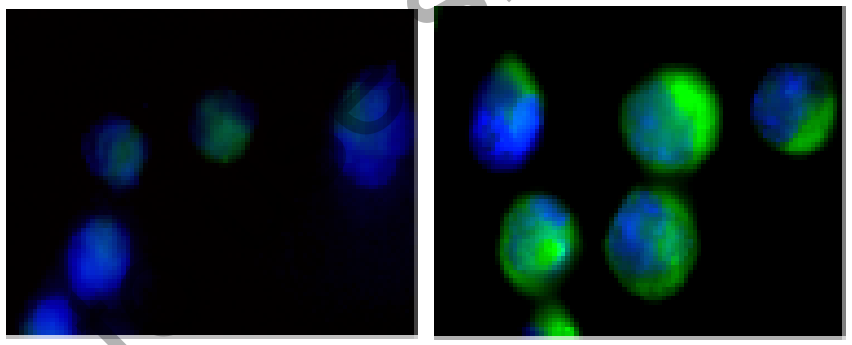
Non small cell lung cancer cells



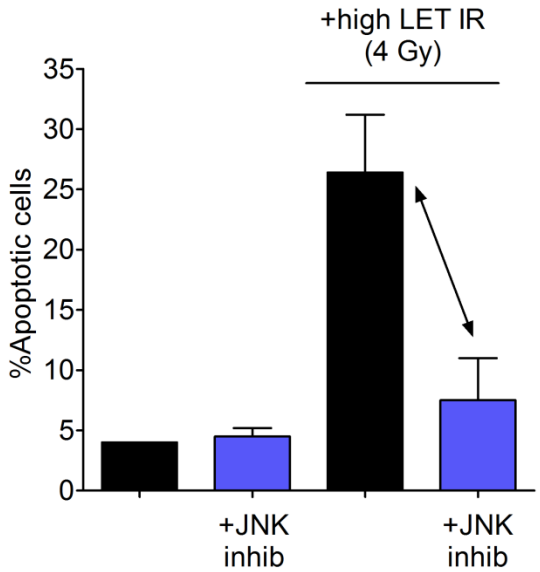


UNT

high LET IR, 4 Gy, 16h

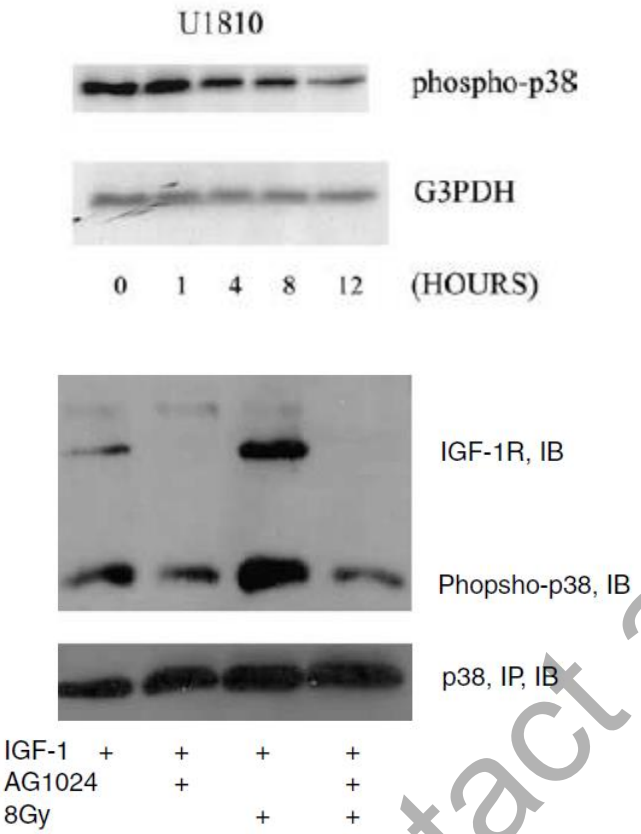


pJNK1/2



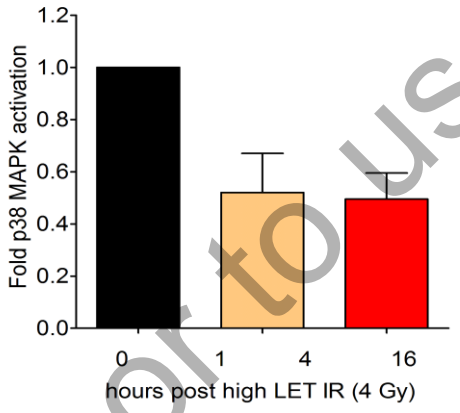
High LET but not low LET IR impairs a p38MAPK-IGF-1R survival signaling loop

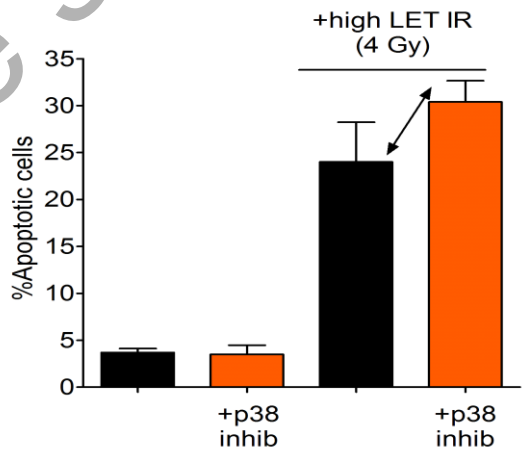
NSCLC, Low LET IR (8 Gy)



Refs: Viktorsson et al., Exp. Cell Research, 2003, Cosaceanu, D, et al., Oncogene, 2007

NSCLC, High LET IR (4Gy)





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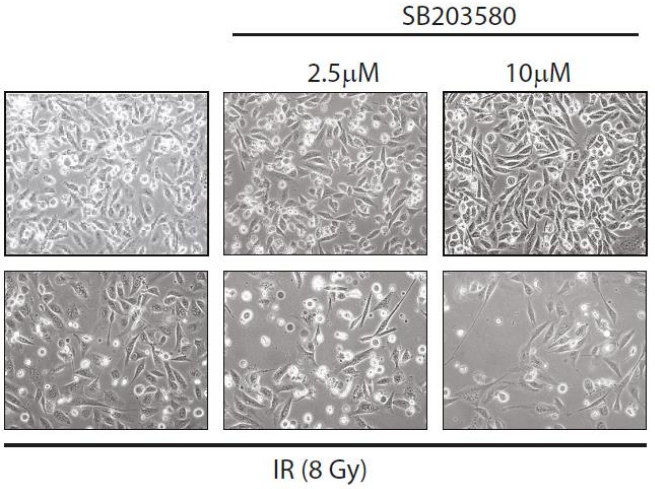
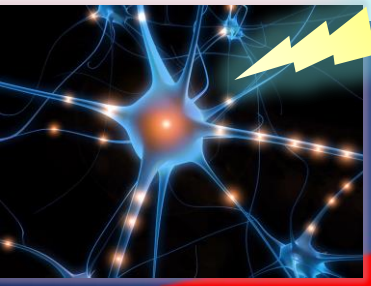


High LET IR can bypass different DNA repair events- what about other signaling cascades ?



Activation of growth factor receptors

EGFR, IGFR, PDGF

DNA damage surveillance

cell survival signalling

p53

ATM

Cell cycle regulation

p21

Chk1/Chk2

cell death signallning

Activation of DNA-repair

Ku70 DNA-PKcs

Ku80

DNA DSBs:
NHEJ, HR
Base damages:
BER
Crosslinks: MMR

Apoptosis
Mitotic catastrophe
Necrosis

Correct DNA repair

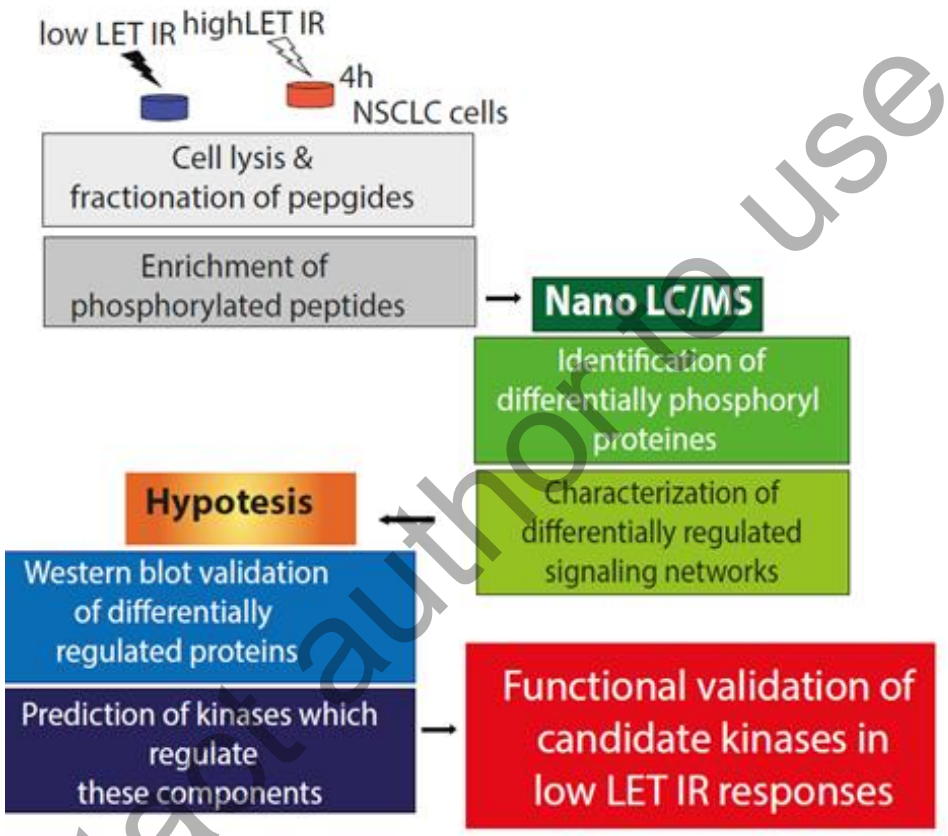
Incorrect DNA repair

Cell survival

Cell with mutation

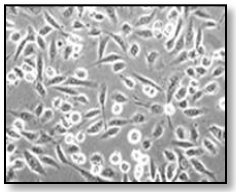
Cell death

Can we by phosphoproteomic analysis identify signaling events of importance for cell death effects after high LET radiation ?

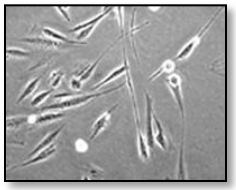


Ståhl, S. Mamede-Branca, R., Mohanty, C., Zielinska-Chomej, K., Efazat, G., Juntti, T., Tu, J, Hååg, P., Stenerlöw, B., ***Lewensohn, R****., Lethio, J. & Viktorsson, K (2014). Phosphoproteomic profiling of high and low LET irradiated Non small cell lung cancer cells reveals differences in growth factor signalling cascades and indicate a role of p38MAPK and GSK3β in low LET radiotherapy cellular response. In manuscript*

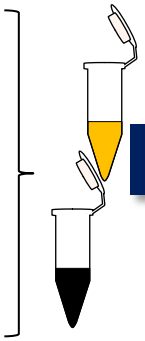
Phosphoproteomic profiling method



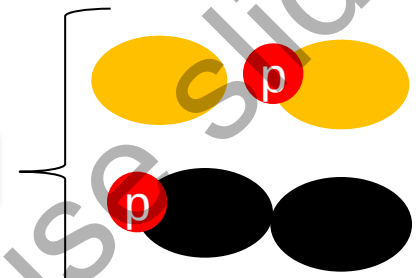
+ low LET IR



+ high LET IR

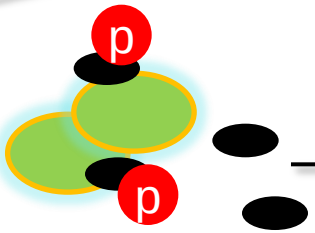


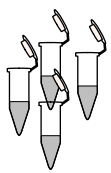
Total cell extracts

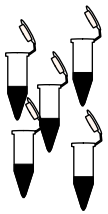


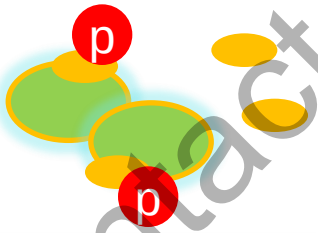
Trypsination into peptides

SCX-fractionation



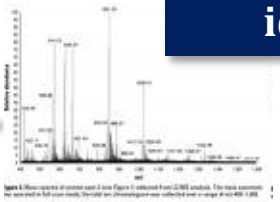




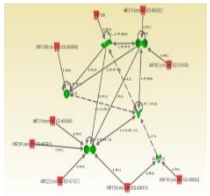


TiO_2 Enrichment of phospho-peptides

Nano-LC MS/MS



MS spectra & Protein identification

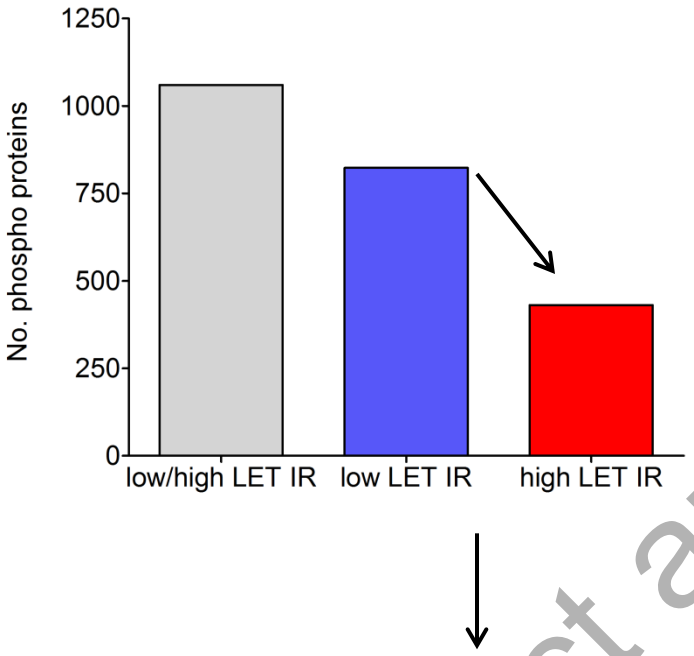


Pathway Analysis

Validation of candidate phosphoproteins

Decreased number of phosphoproteins identified in response to high LET IR- impaired growth factor signaling ?

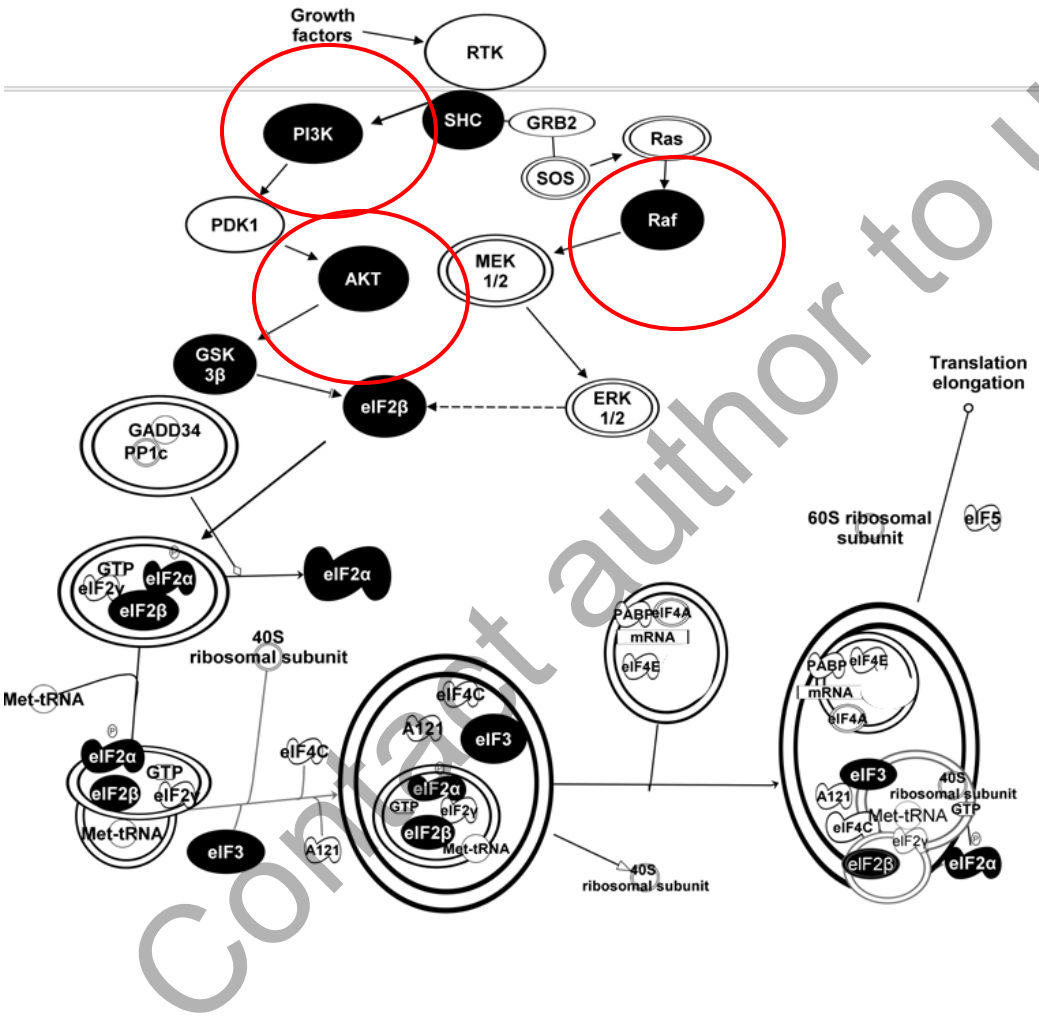
Inhibition of signal transduction In response to high LET IR



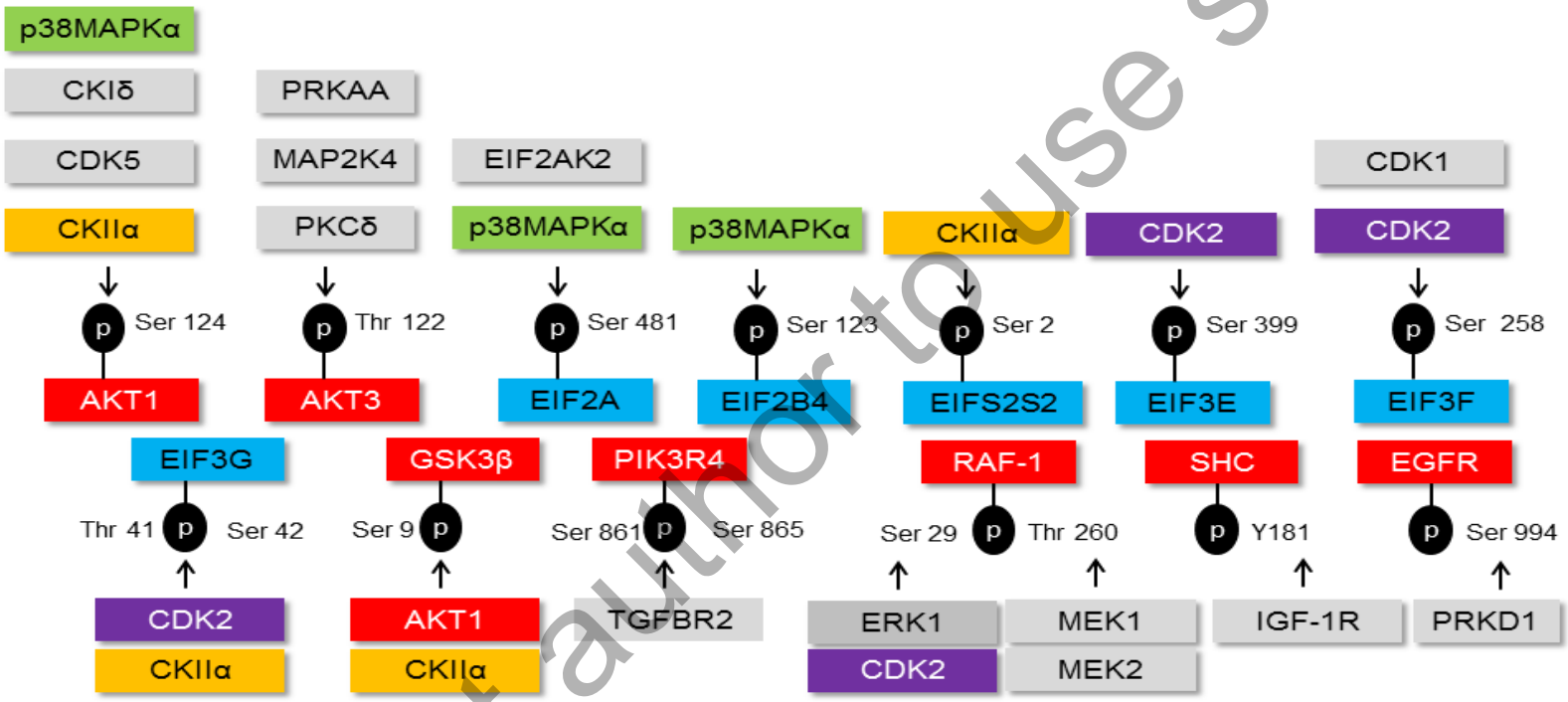
What pathways are differentially regulated ?

PI3K/Akt/GSK3B signaling is inactivated by high LET IR-blockade of protein translation

Black marked does not show phosphorylation after high LET IR in NSCLC cells



p38MAPK, CKII, AKT1 & CDK2 –signaling pathways of importance for cell death trigger in response to radiation in NSCLC cells ? inhibition av celldöd vid låg LET IR ?

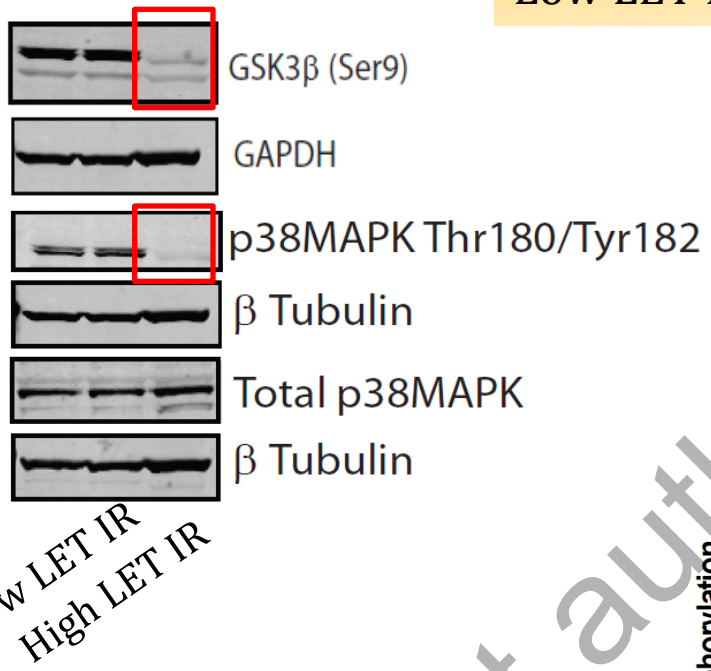


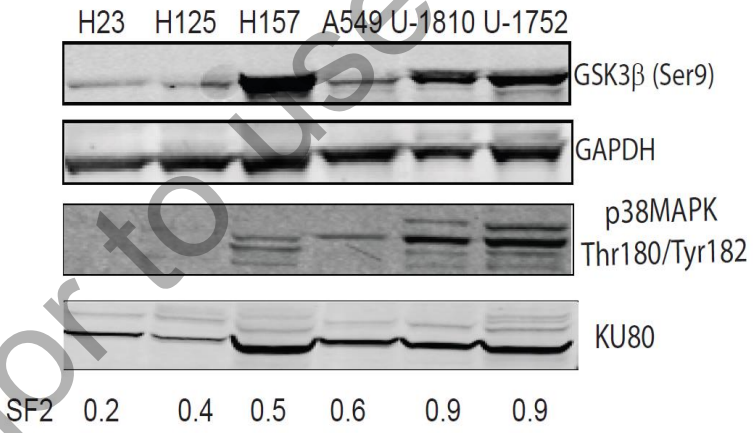
GSK3β & p38MAPK →

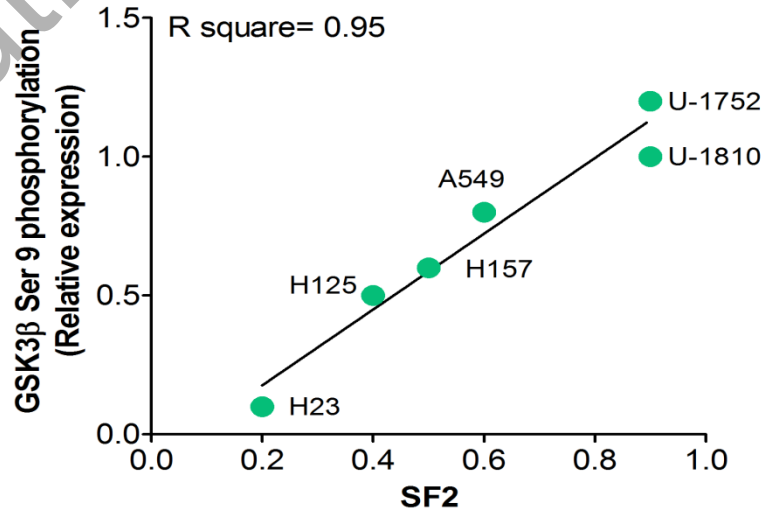
Biologic validation in low LET IR response

GSK3β & p38MAPK drives low LET IR resistens & are inactivated by high LET IR

Low LET IR resistant cells have higher GSK3B & p38 activity

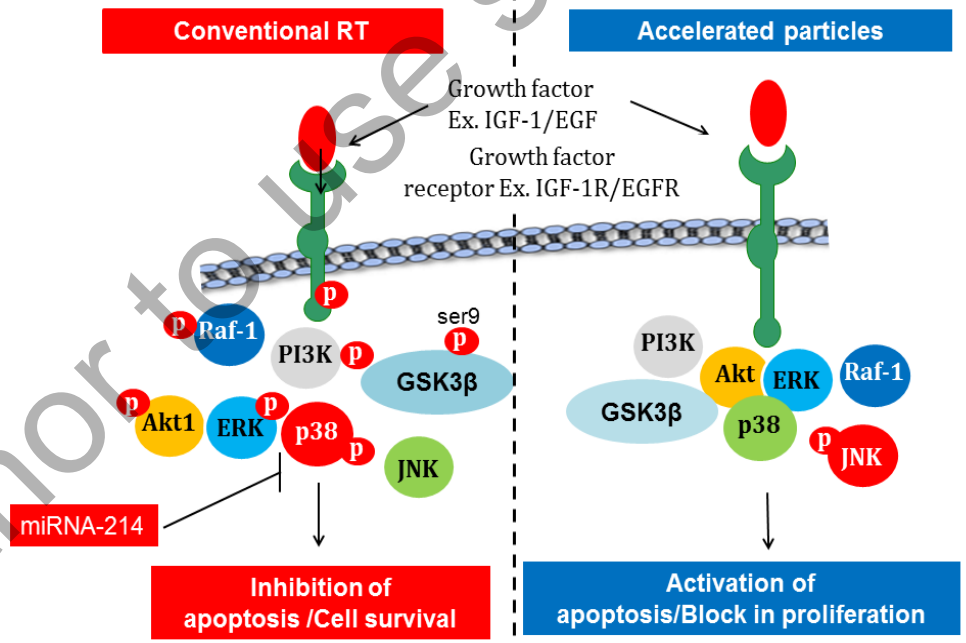




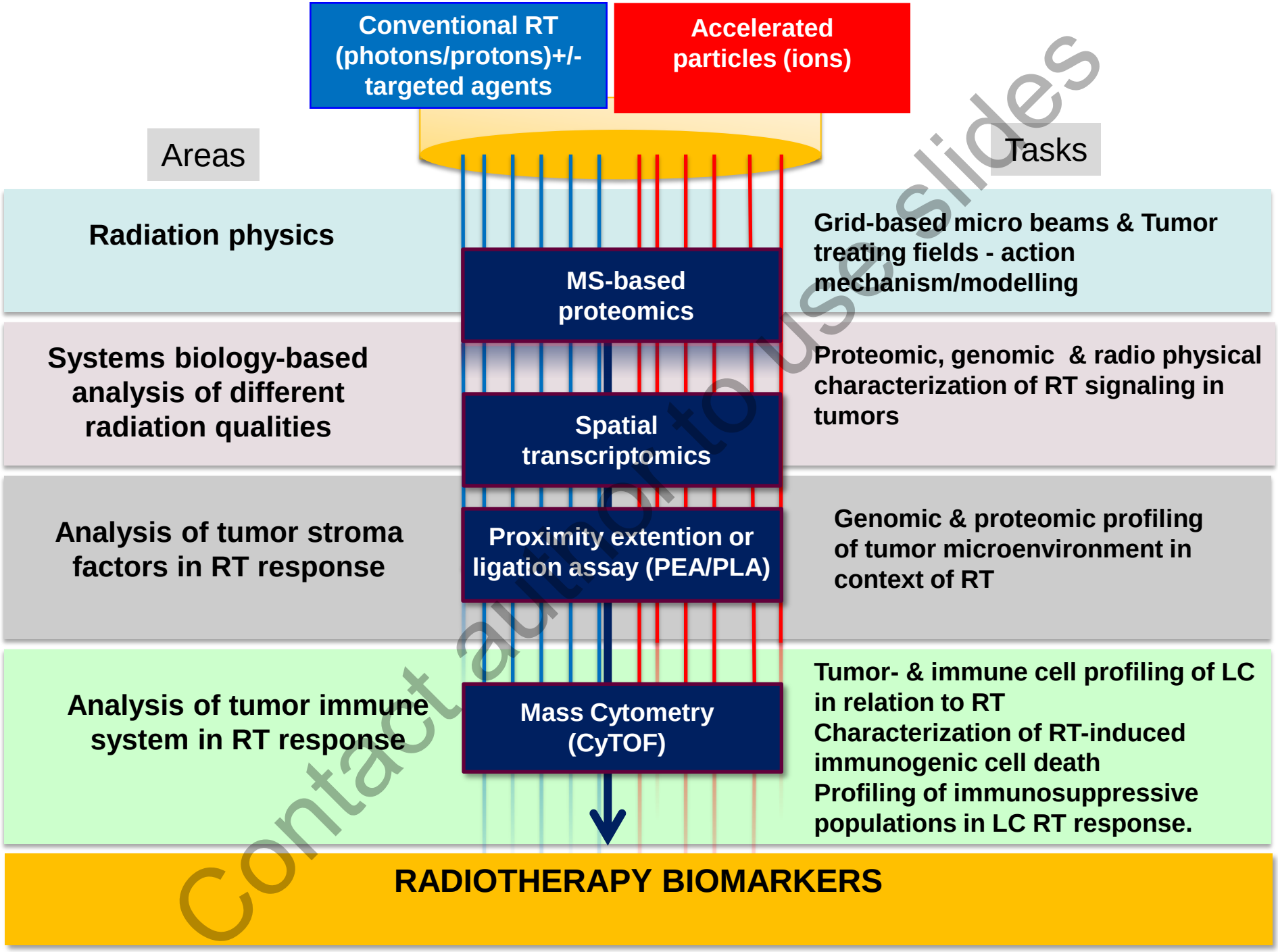


Summary of pathways of importance for low and high LET IR response in tumor cells

- High LET IR but not low LET IR causes more **complex DNA-breaks** and induces **apoptosis** in NSCLC cells indicating different signalling pathways.
- Total and phosphoproteomic analyses revealed signaling differences in response to low and high LET IR
  - **JNK-** a pro-death pathway
  - **p38MAPK/GSK3B-** survival pathways.



To whom should we apply high LET IR- Biomarkers to enable patient selection required ?



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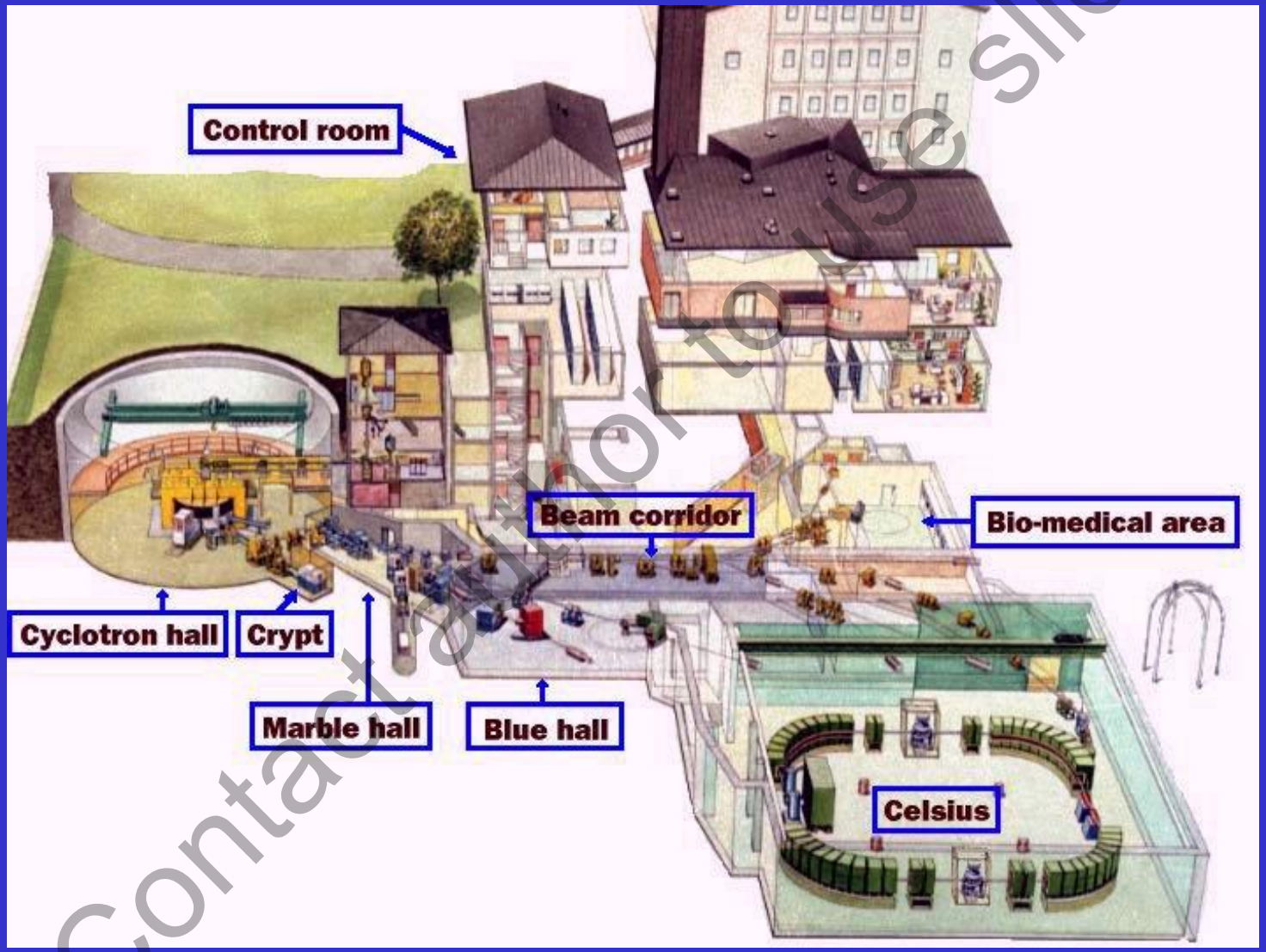

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