

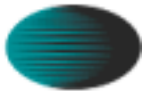
Biology and Radiobiology in HNSCC

Updates and potential Clinical Applications

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Dept. of Radiation Oncology
Eberhard-Karls University Tübingen

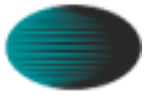




Update on the radiobiology in HNSCC

..... with respect to

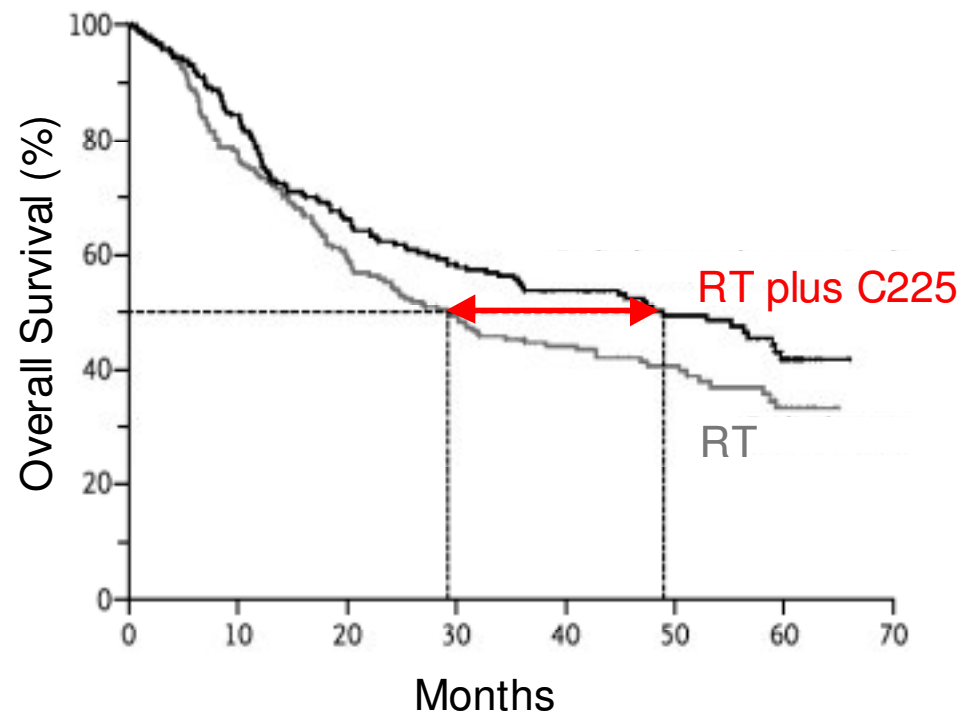
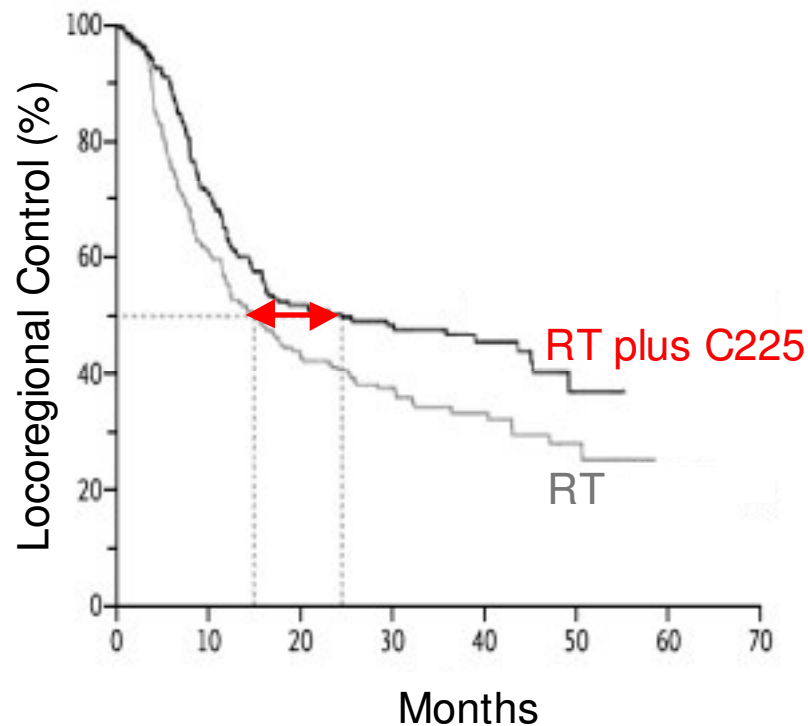
- EGFR
- EGFR and Hypoxia
- Autophagy



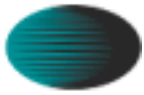
Relevance of EGFR inhibition by C225 for radiotherapy

Bonner et al. New Engl J Med 2006

424 H&N tumor patients : 213 RT+**Cetuximab (C225)** / 211 RT alone



... study was planned around 1998 and started in 2000, at a time when the mechanisms of how EGFR mediates cellular radiation responses was not clear at all !



Frequencies of solid tumors with mutated or overexpressed EGFR

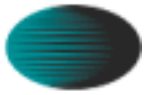
Solid tumors presenting mutated or overexpressed EGFR

- H&N 80-100%
- Renal 50-90%
- Breast 14-91%
- Esophageal 43-89%
- Prostate 40-80%
- NSCLC 40-80%
- Colorectal 25-77%
- Gastric 33-74%
- Ovarian 35-70%
- Glioma 40-63%
- Pancreatic 30-50%
- Bladder 31-48%

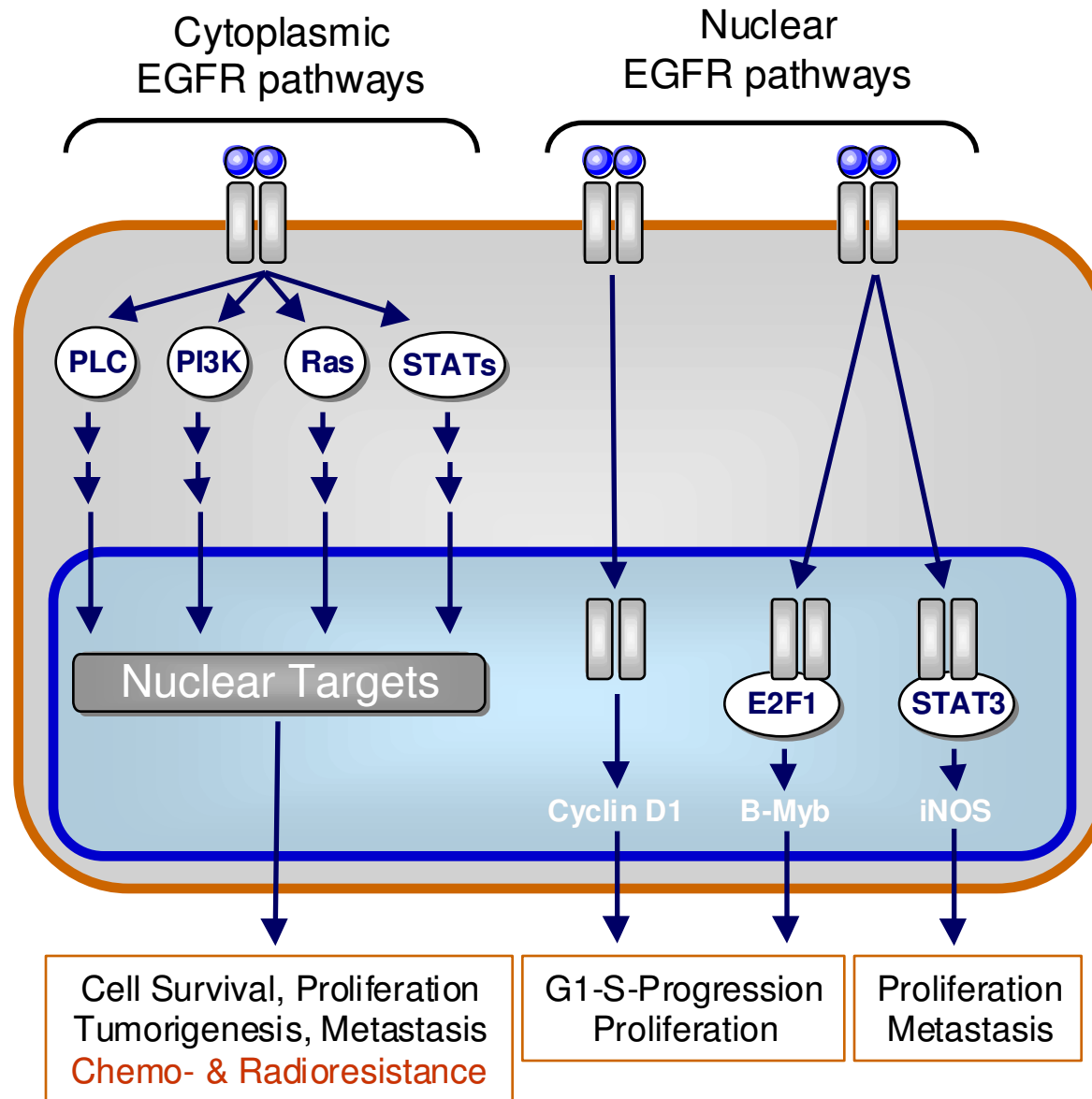
Mutation/overexpression is associated with ...

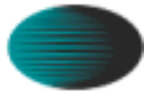
- enhanced receptor signaling
- massive tumor growth
- enhanced invasive and metastatic potential

...and is generally correlated with resistance to chemo-/ radiotherapy

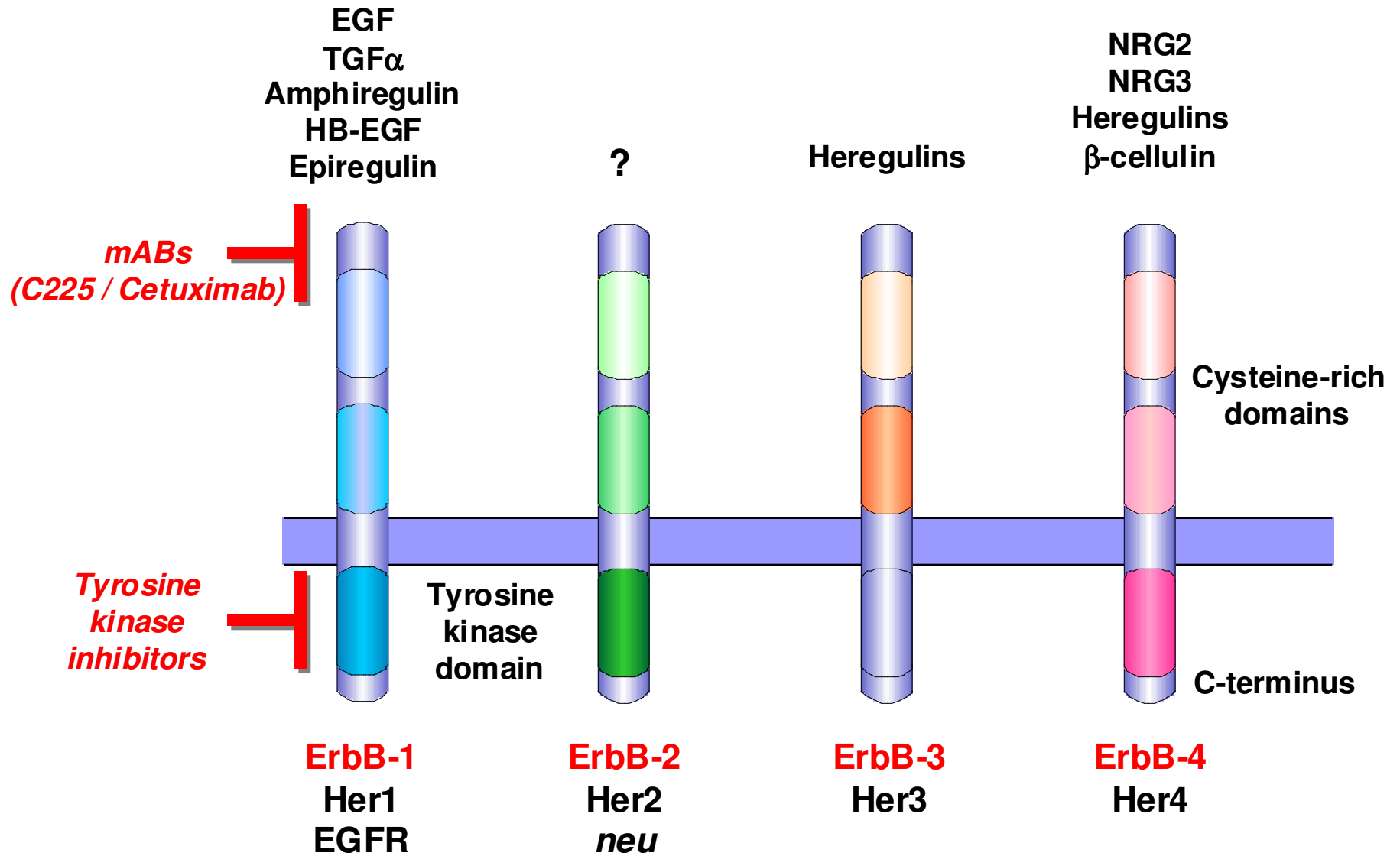


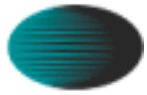
Importance of EGFR signaling in oncology



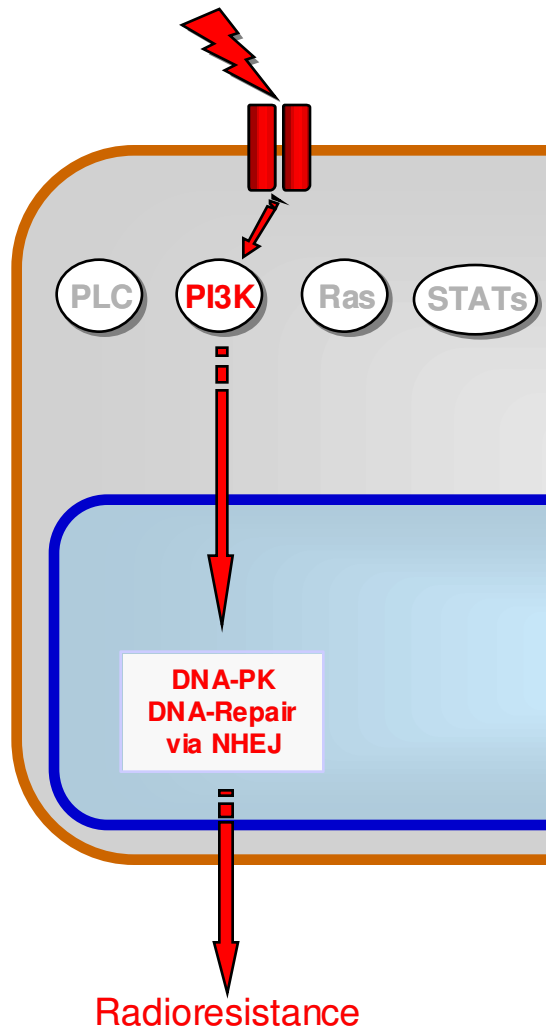


ErbB-receptor family and its ligands





Radiobiological properties of EGFR signaling

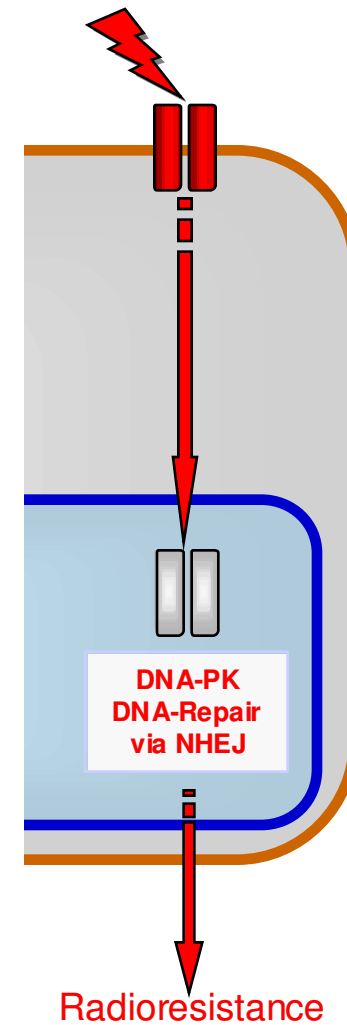


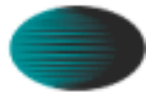
Güven et al.
J Biol Chem 2001
Genes Chromos Cancer 2003

Dittmann et al.
J Biol Chem 2005
Radiother Oncol 2005
Radiother Oncol 2007
Int J Rad Oncol Biol Phys 2008
Radiother Oncol 2008
Mol Cancer 2008
Radiother Oncol 2009 subm.
J Biol Chem 2009 subm.

Toulany et al.
Radiother Oncol 2005a
Radiother Oncol 2005b
Clin Cancer Res 2006
Mol Cancer Res 2007
Mol Cancer Ther 2008
DNA Repair 2008
Radiother Oncol 2009 subm.
Cancer Res 2009 subm.

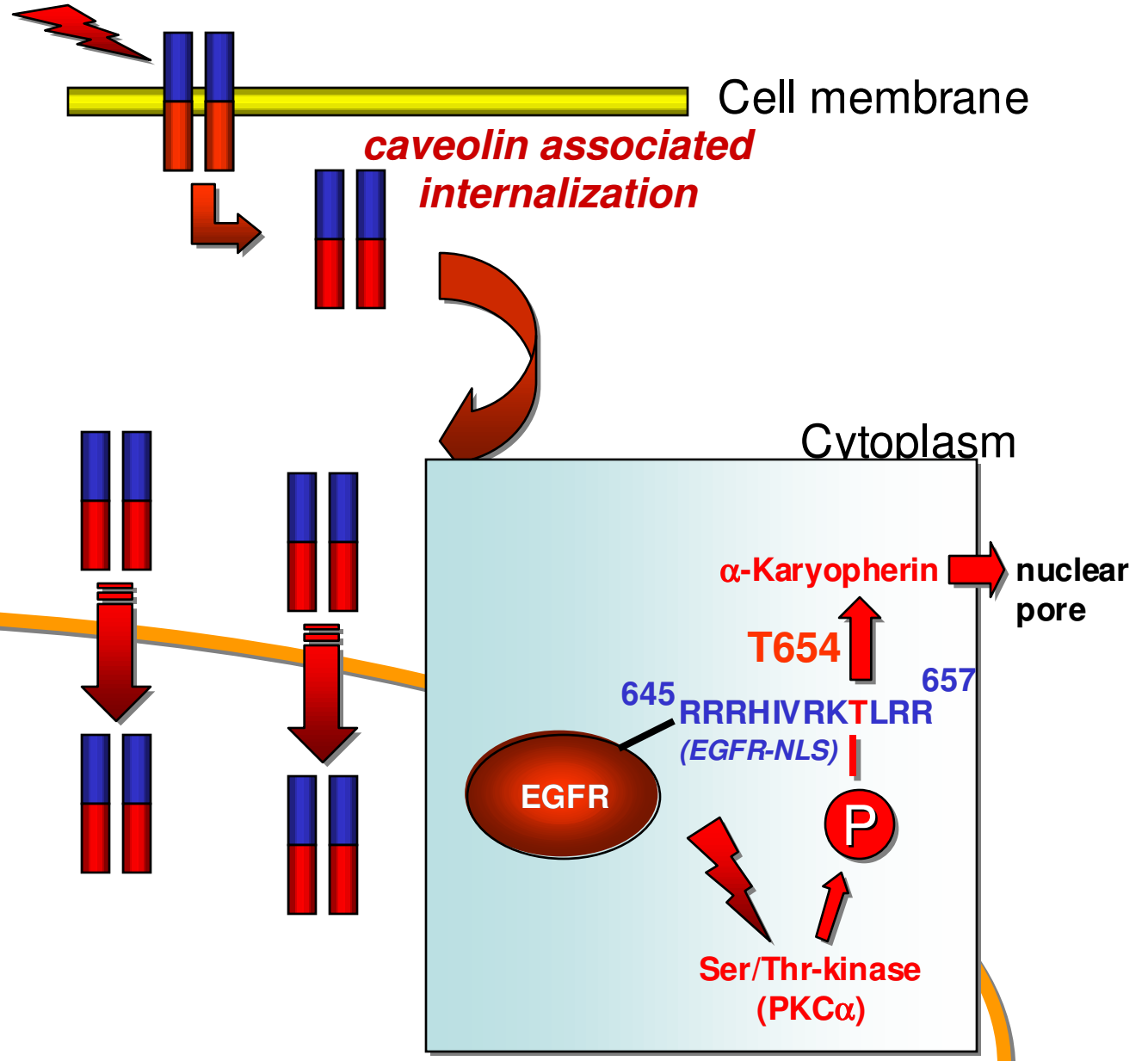
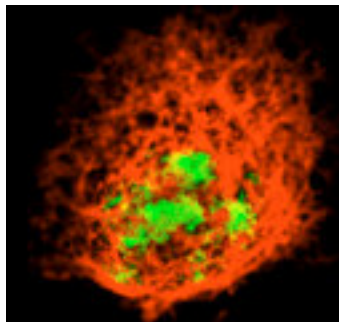
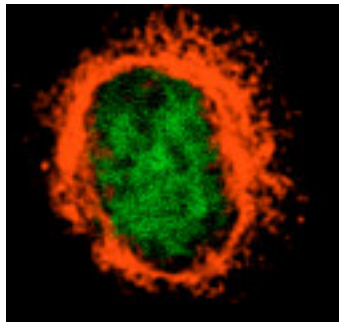
Rodemann et al.
Sem Rad Oncol 2007
Int J Rad Biol 2007

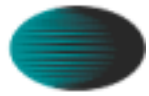




How is IR-induced nuclear accumulation of EGFR achieved ?

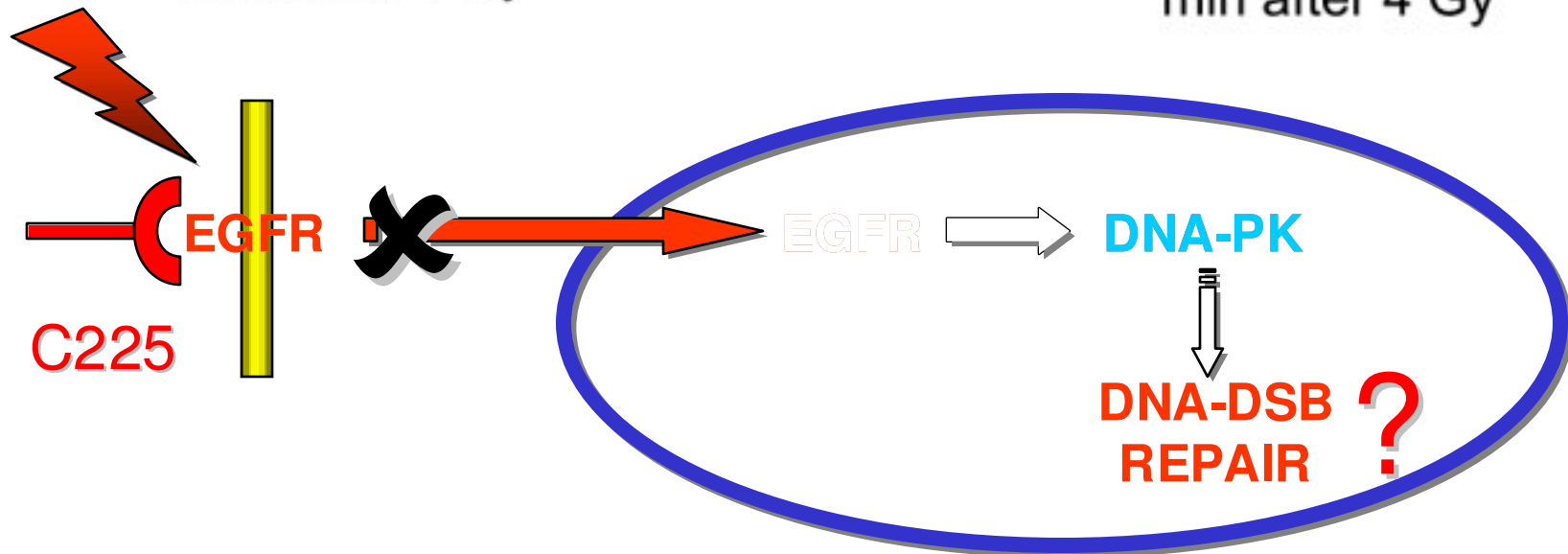
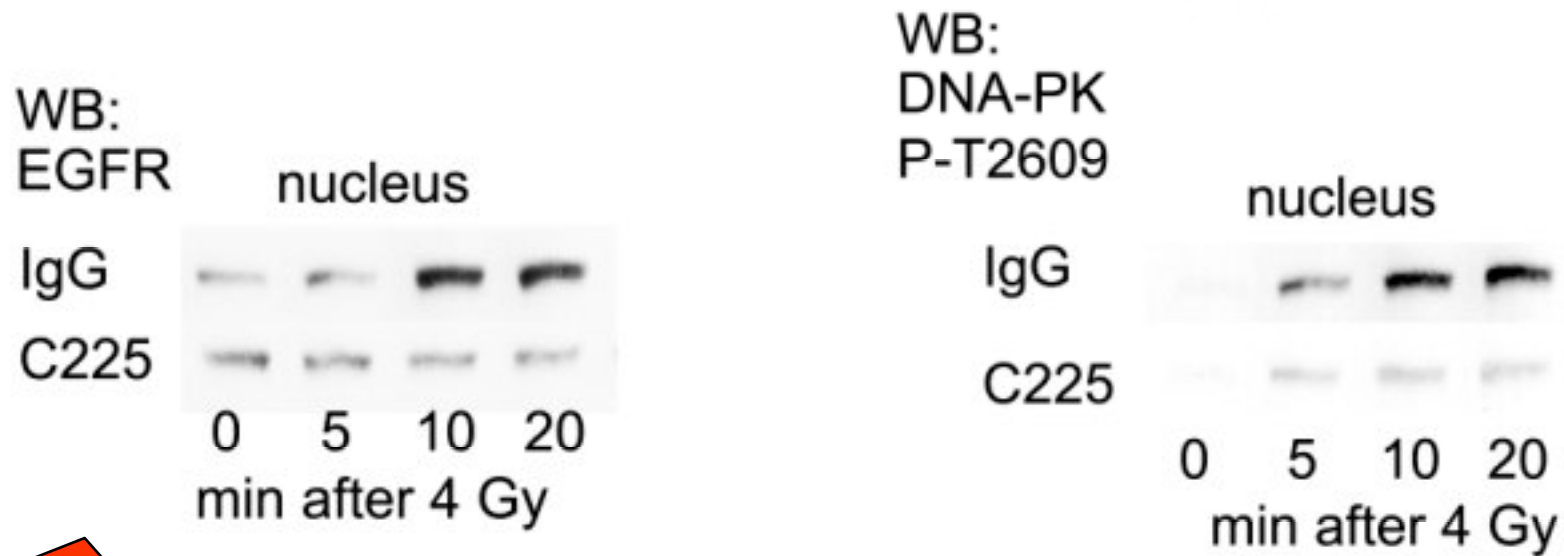
Dittmann et al.
J Biol Chem 2005
Radiother Oncol 2005
Radiother Oncol 2007
Radiother Oncol 2008
Mol Cancer 2008

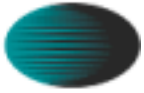




C225 prevents radiation-induced nuclear accumulation of EGFR and activation of DNA-PK

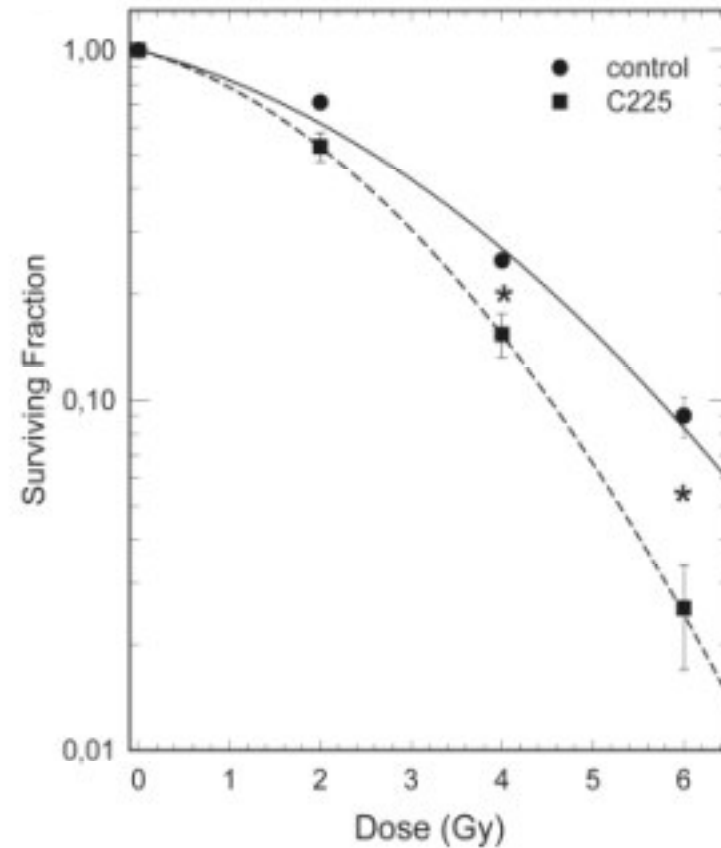
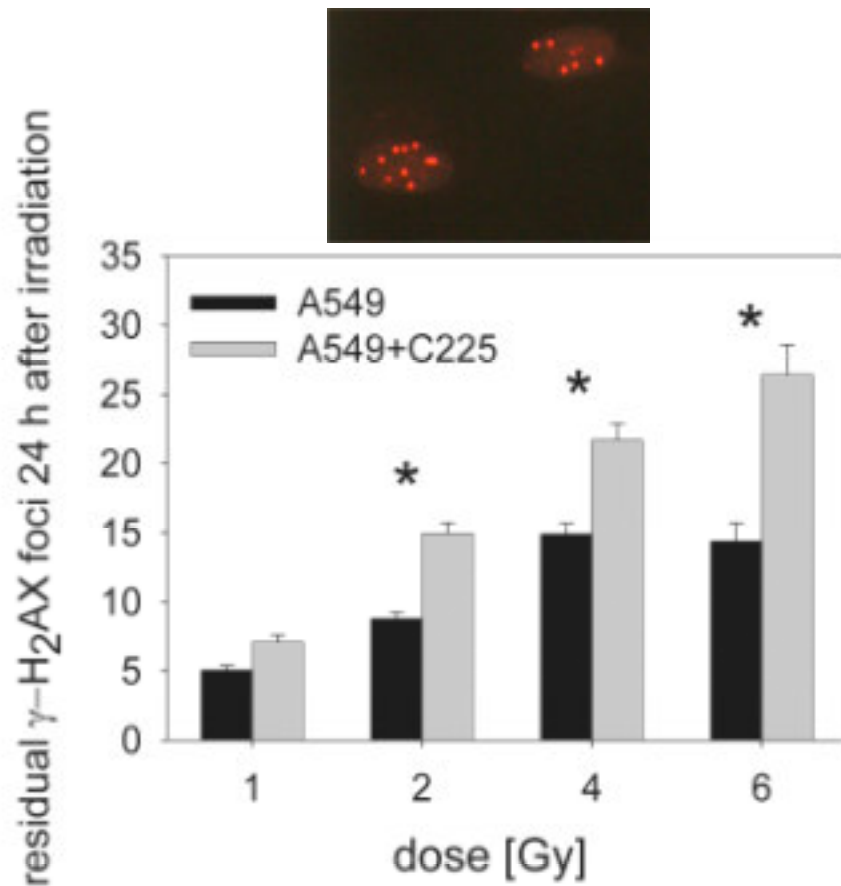
Dittmann et al. J Biol Chem 2005



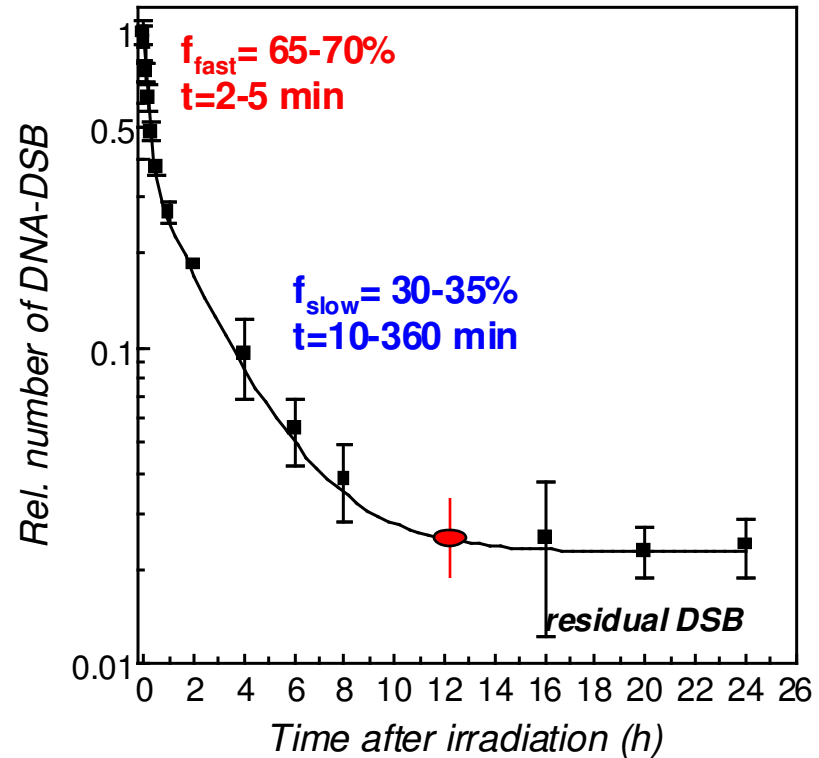
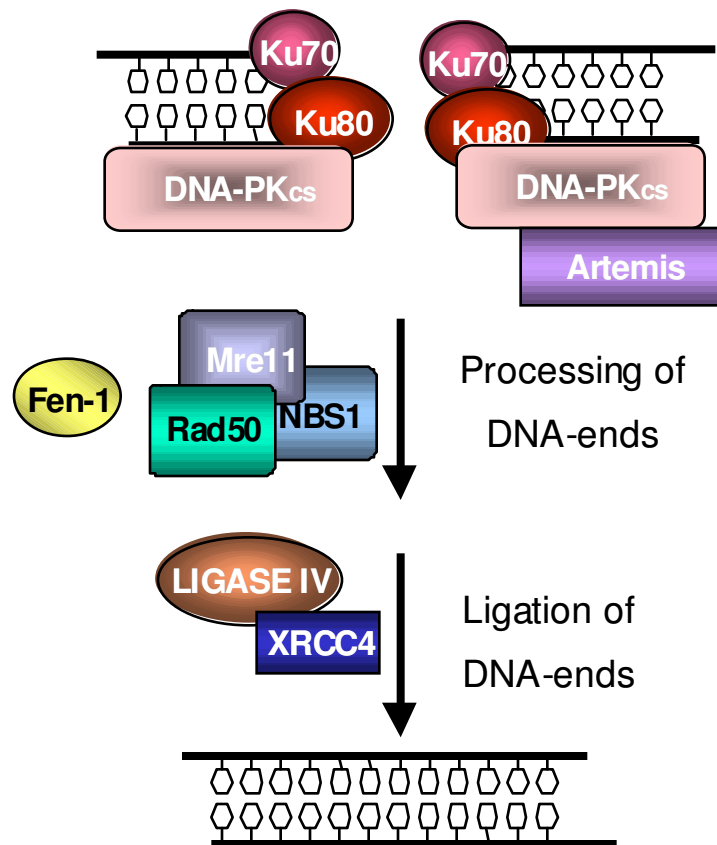


C225 mediates impaired DNA-DSB repair and enhances radiation sensitivity

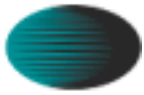
Dittmann et al.
J Biol Chem 2005
Radiother Oncol 2005



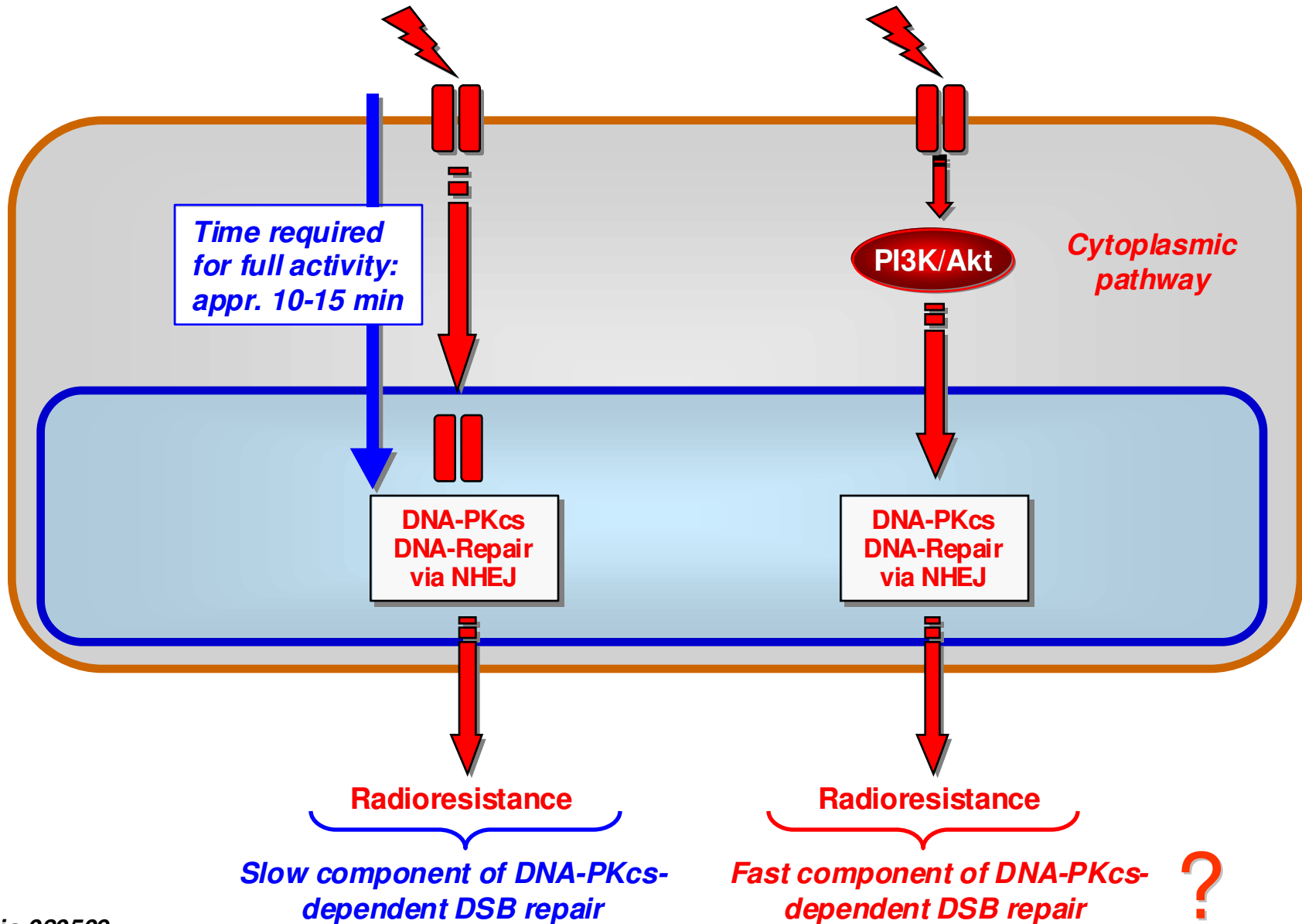
DNA-DSB repair by NHEJ and its kinetic



- There is *fast* and *slow* repair of DNA-DSB
- Repair is finished after about 12 h
- Kinetic of repair is independent of radiation dose

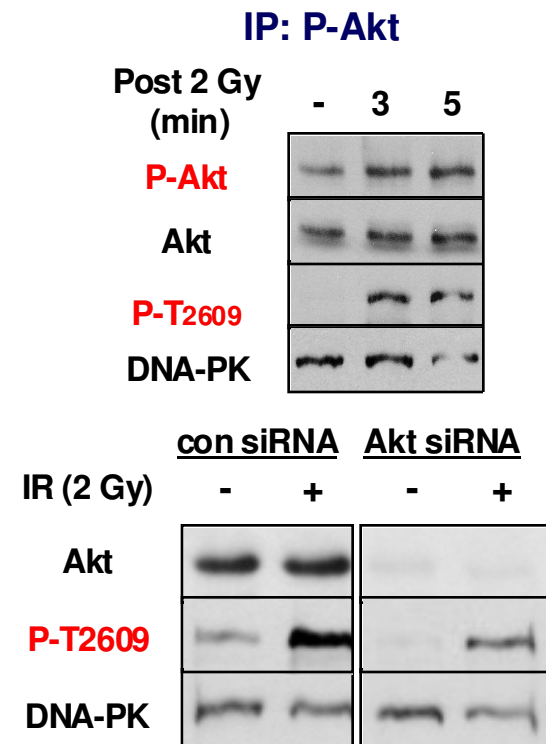
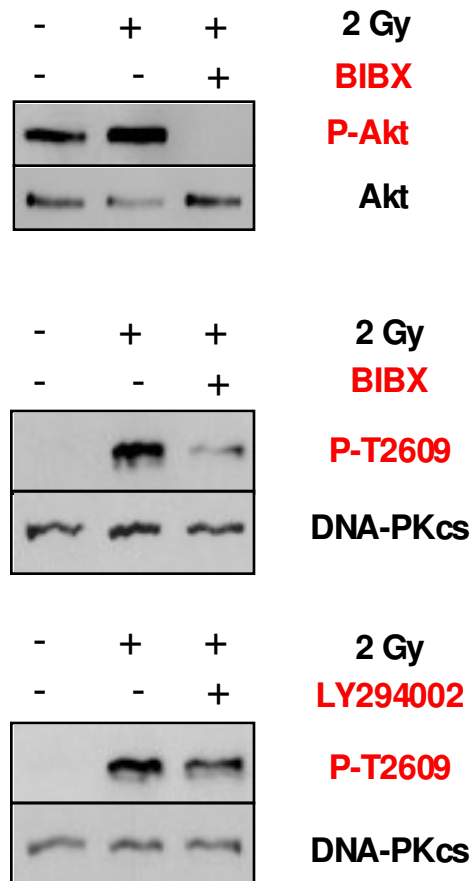
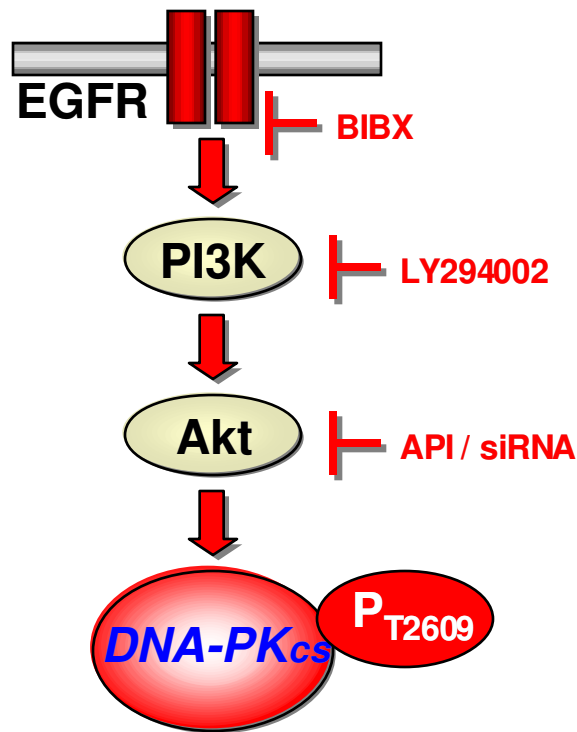


Functions of EGFR-signaling in regulating DNA repair



EGFR-PI3K-Akt signaling is involved in radiation-induced fast activation DNA-PKcs

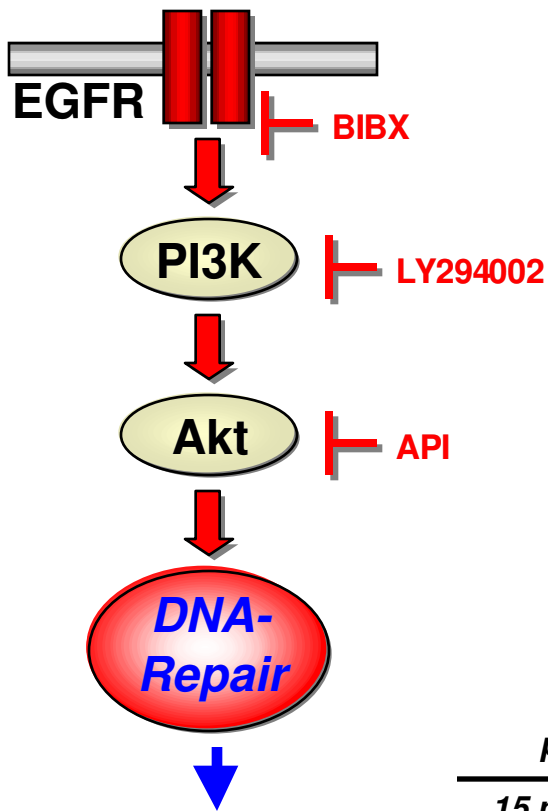
Toulany et al.
Clin Cancer Res 2006
Mol Cancer Ther 2008



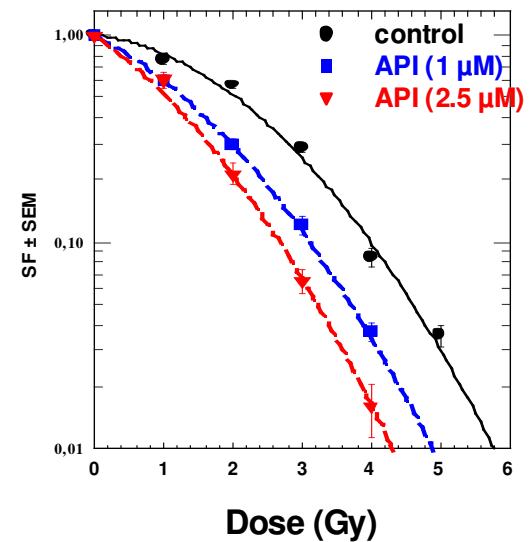
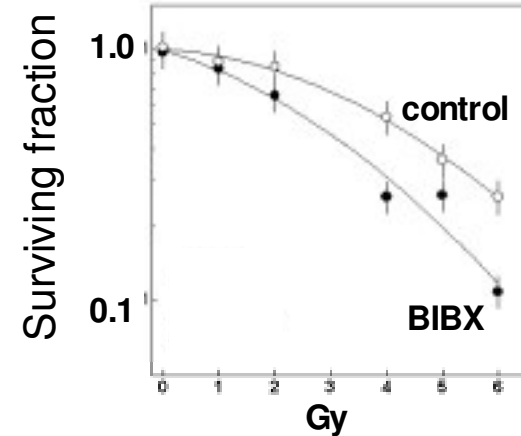
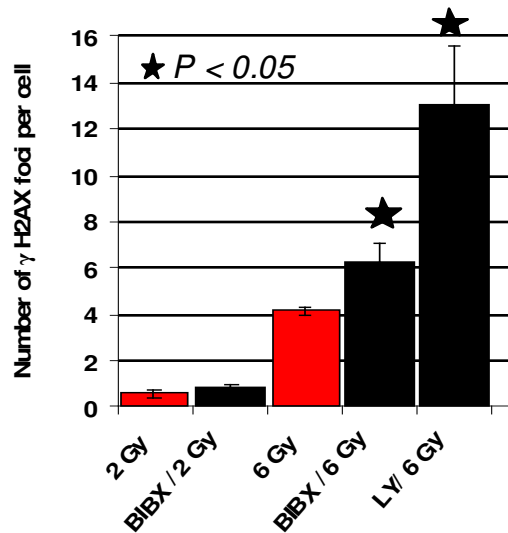
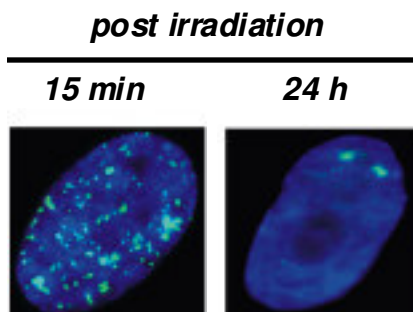
Analyses done 2 min post IR

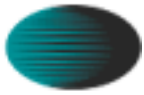
EGFR-PI3K-Akt signaling is involved in radiation-induced DNA-DSB-repair

Toulany et al.
Clin Cancer Res 2006
Mol Cancer Ther 2008

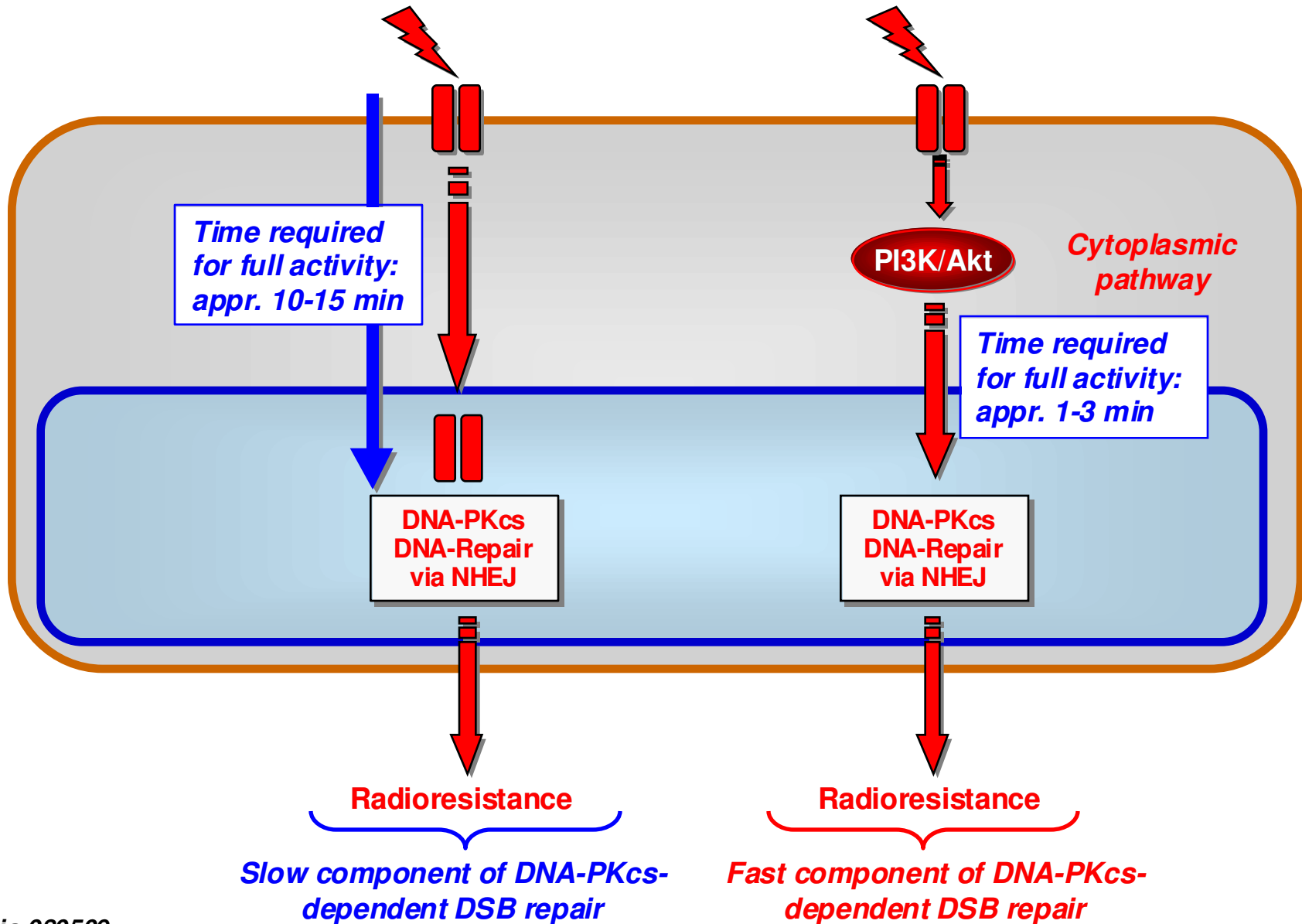


H2AX-Focus-Assay
 detects DNA-DSB

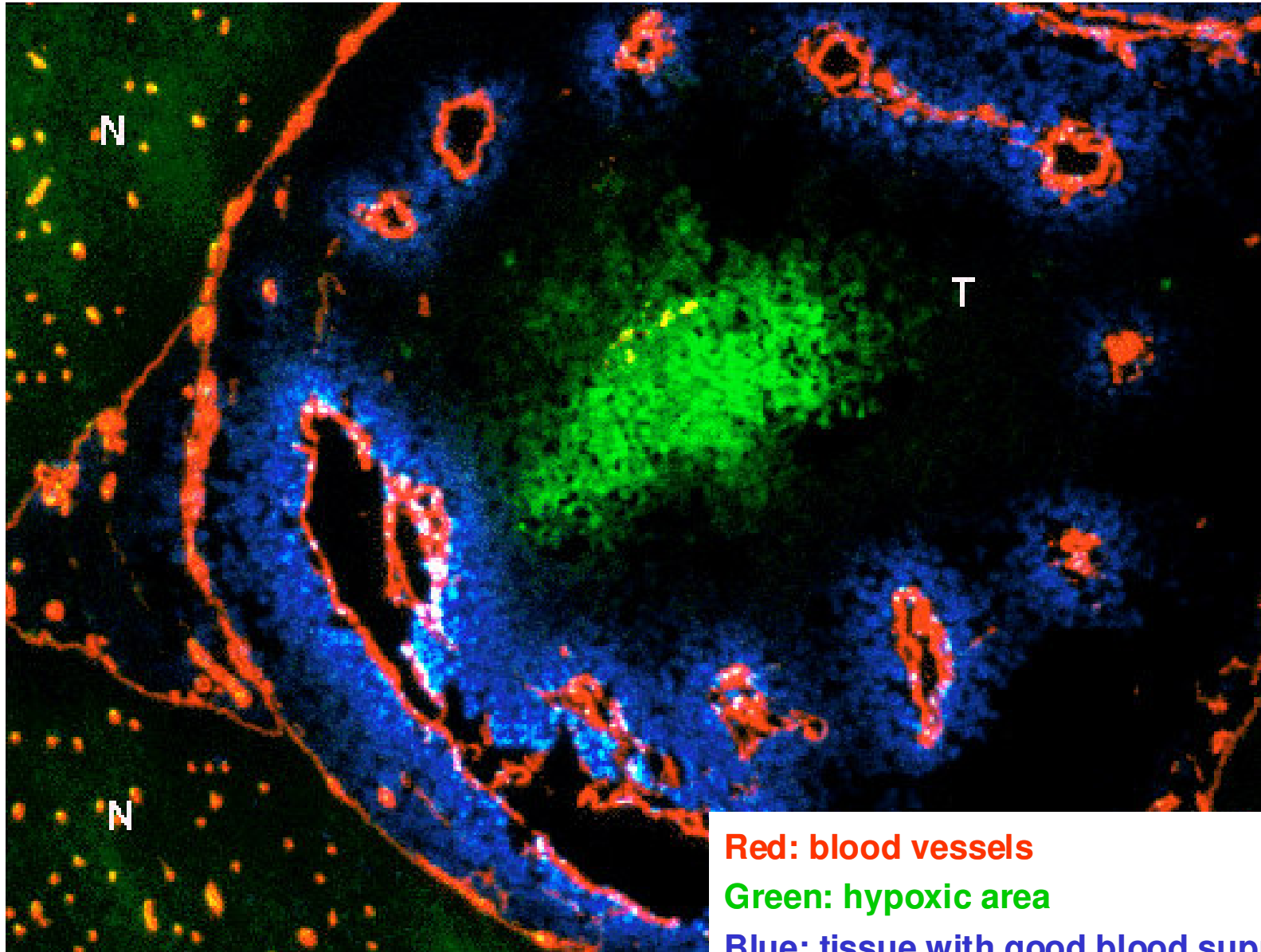




Functions of EGFR-signaling in regulating DNA repair



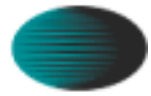
Tumor hypoxia and EGFR expression ?



Red: blood vessels

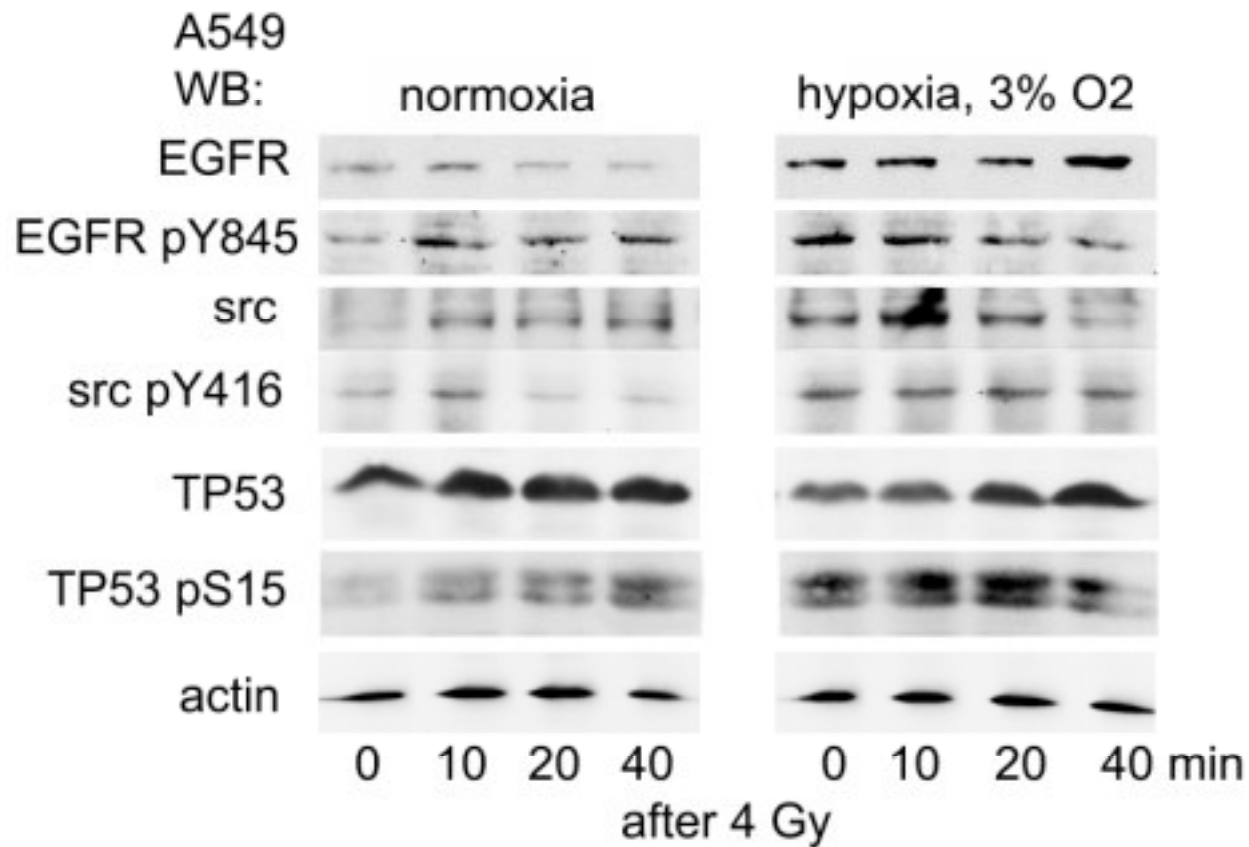
Green: hypoxic area

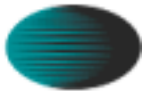
Blue: tissue with good blood supply



EGFR is stabilized and activated by exposure to hypoxia

Pietta et al. unpublished 2009

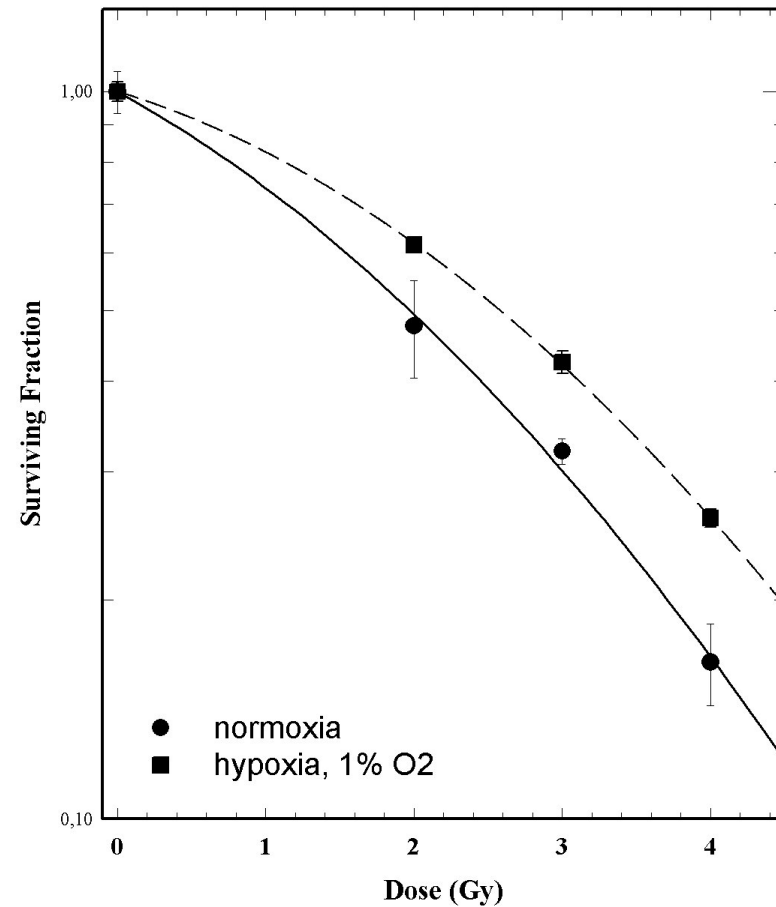
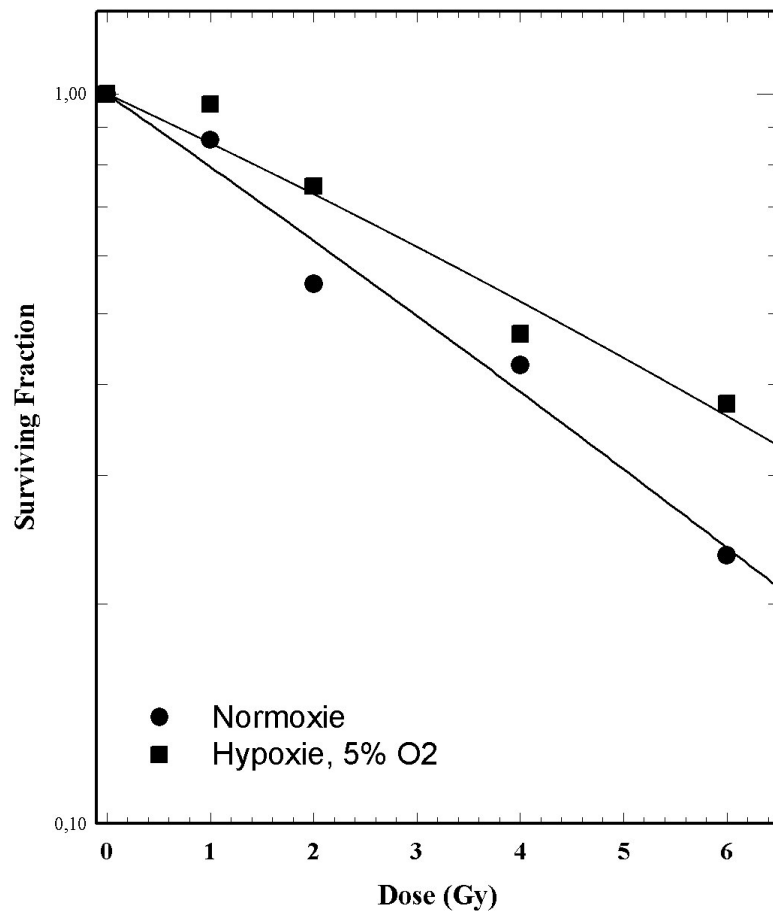


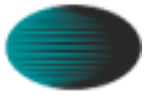


Hypoxia mediates radioresistance

Pietta et al. unpublished 2009

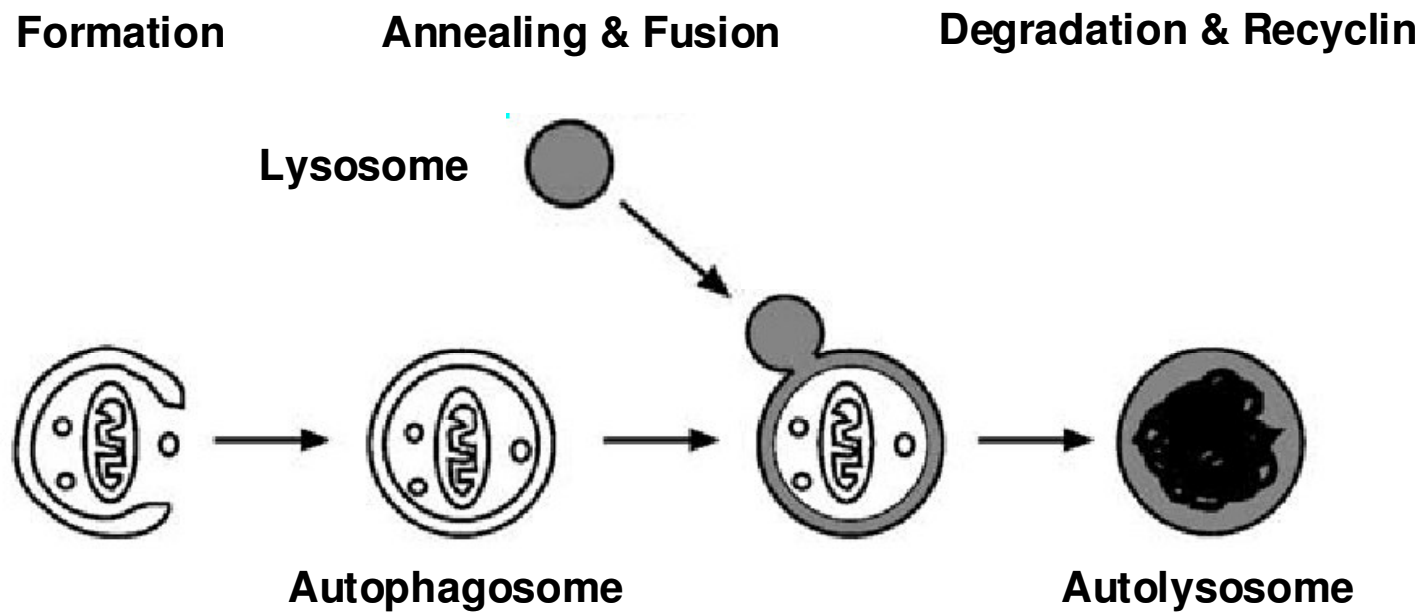
A549 lung cancer cells in vitro

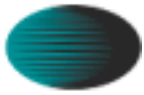




Autophagy – induction and execution

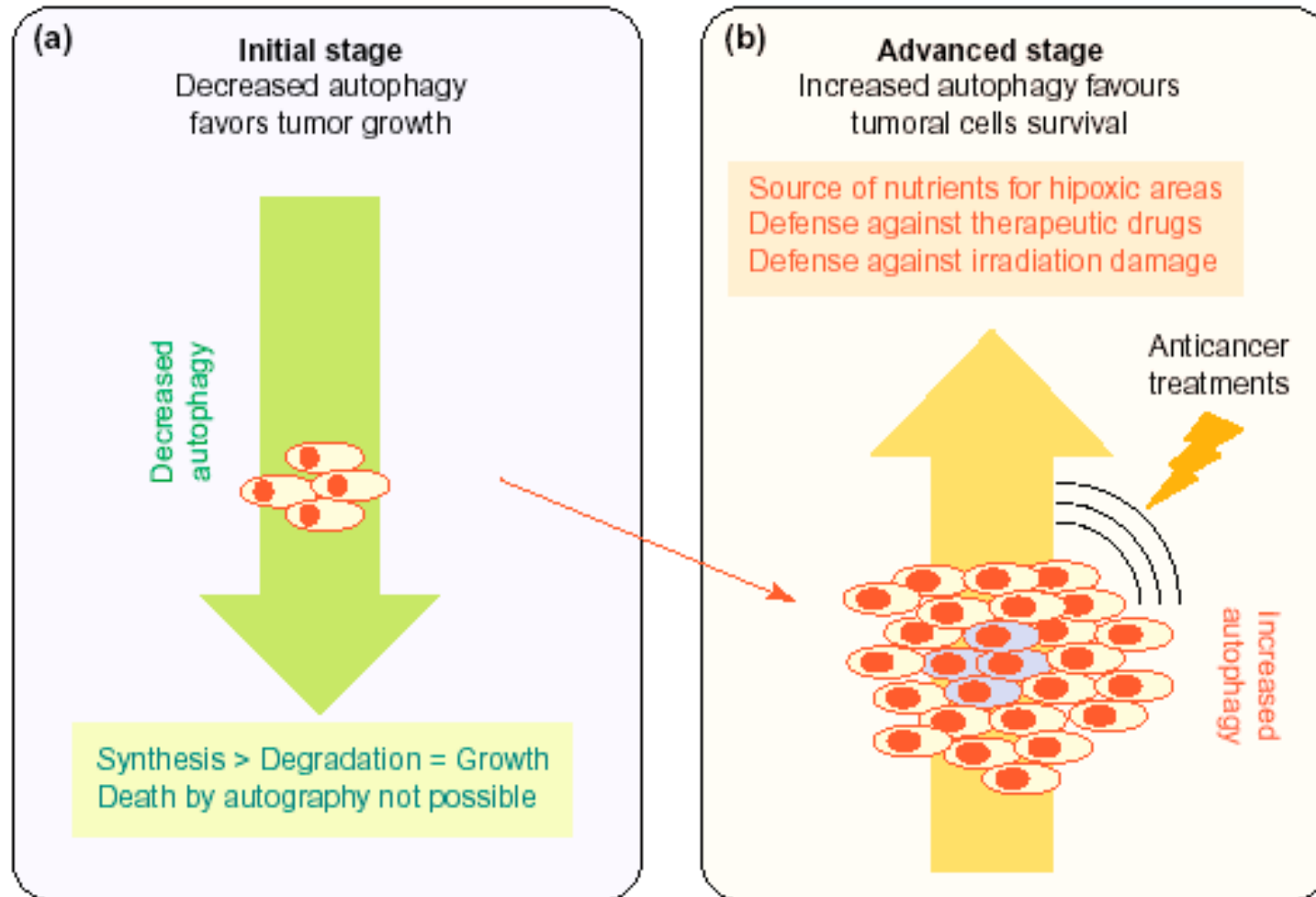
Klionsky and Emr (2000), Mizushima et al. (2003)



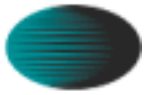


Autophagy – dual role in oncology

Cuervo (2004)

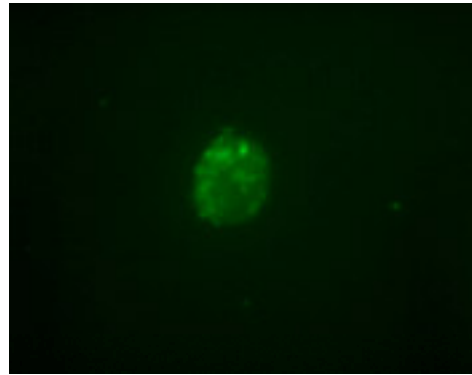


.... thus, inhibition of autophagy should induce radiation sensitivity of tumor cells !

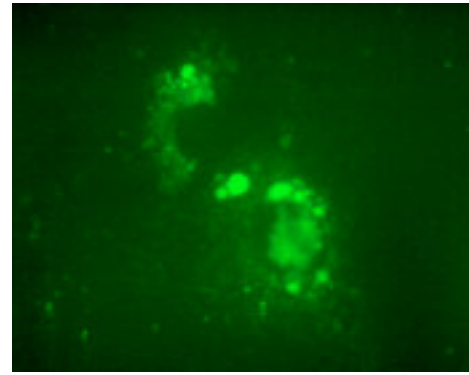


Ionizing radiation induces autophagy

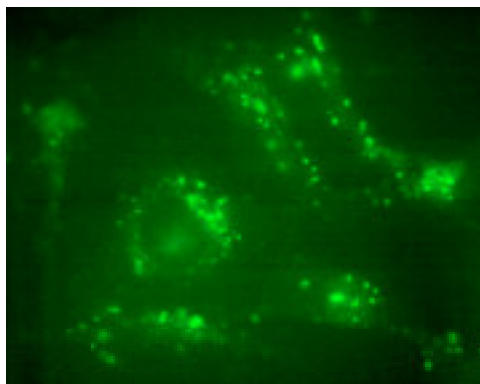
Apel et al. Cancer Res. 2008



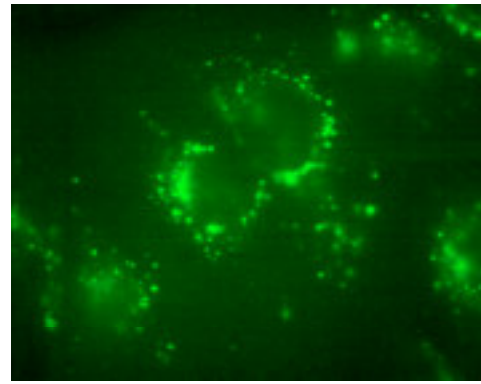
Control
(Untreated / unirradiated)



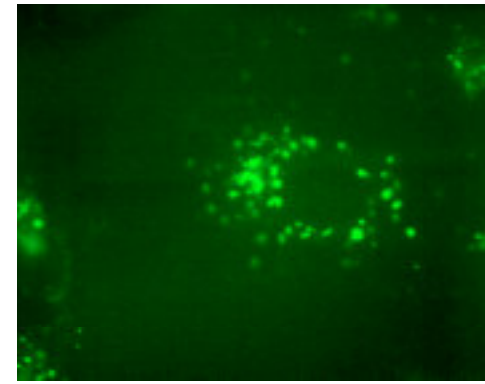
72 h after treatment
with Rapamycin



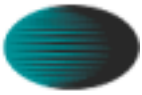
4 h after 2 Gy



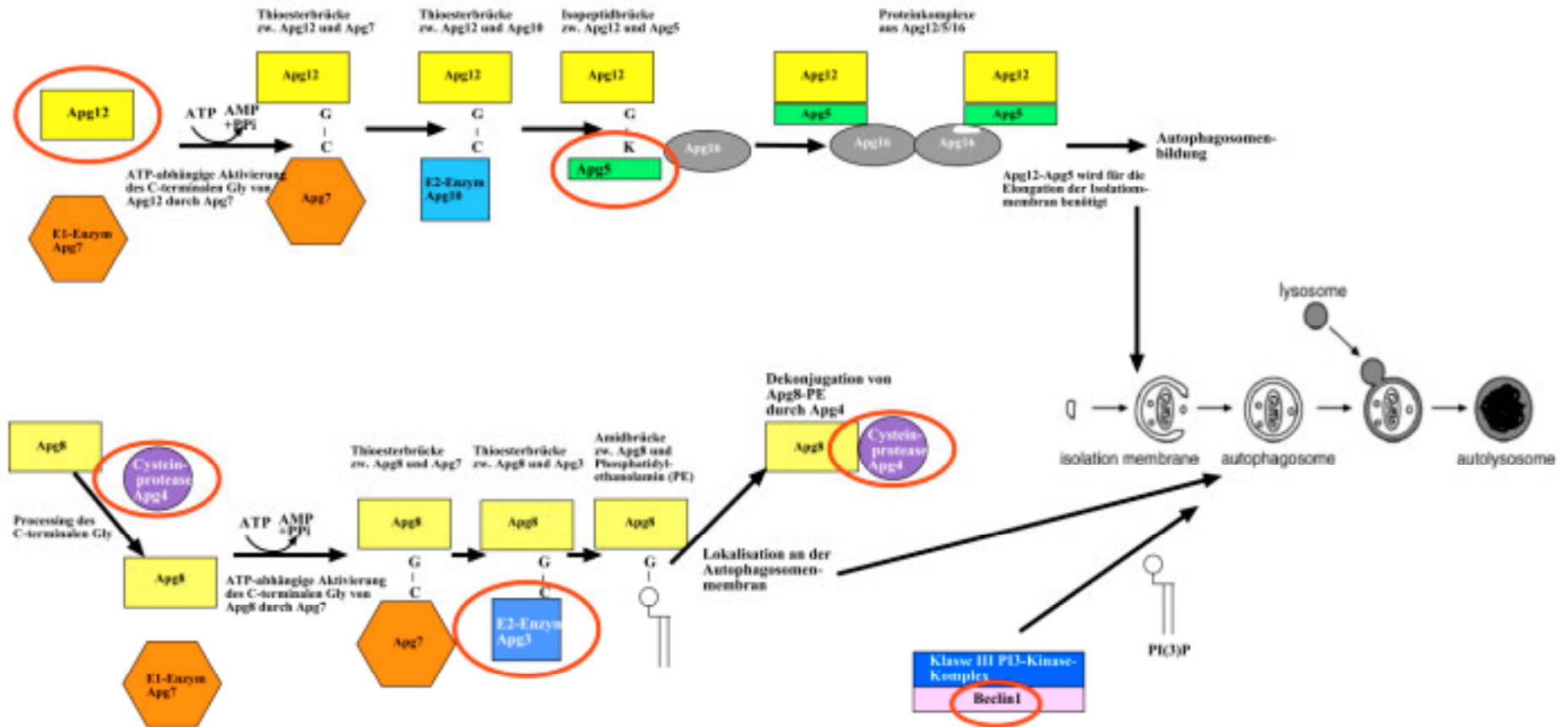
4 h after 4 Gy

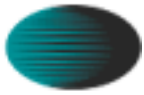


4 h after 10 Gy



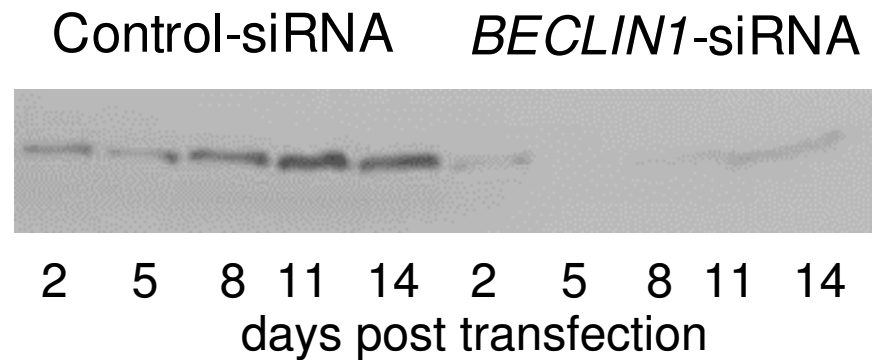
Protein components necessary for autophagosome formation



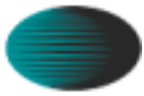


siRNA approach to inhibit autophagy proteins

Apel et al. Cancer Res. 2008



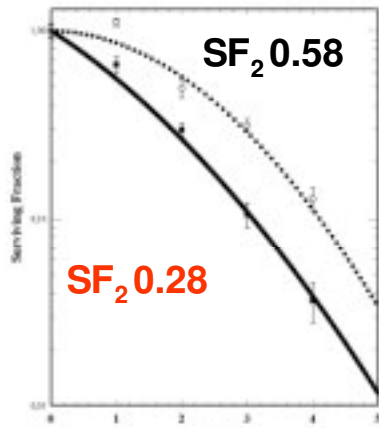
stable inhibition of protein expression for 5-8 days



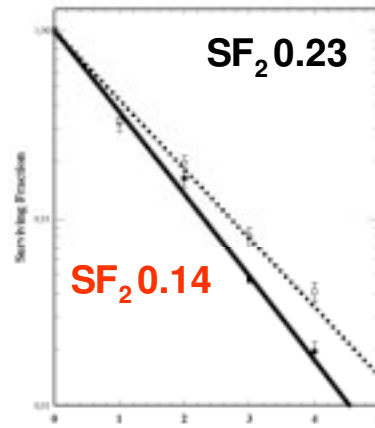
APG4B-siRNA enhances radiation sensitivity in SCC cells in vitro

Apel et al. Cancer Res. 2008

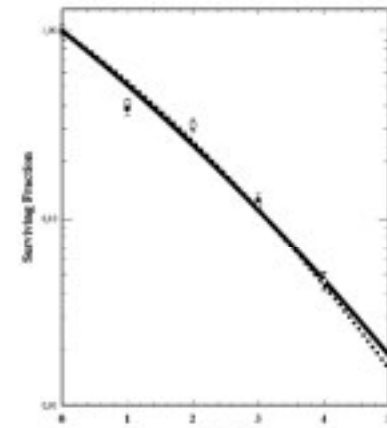
FADU
HN-SCC



HTB35
Cervix-SCC



A549
NSCLC



Single dose
irradiation
(1 - 4 Gy)

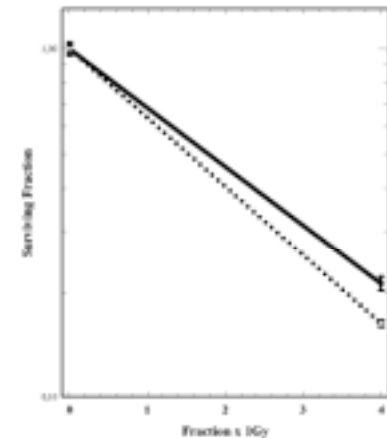
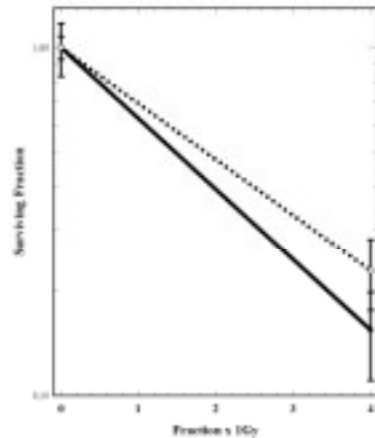
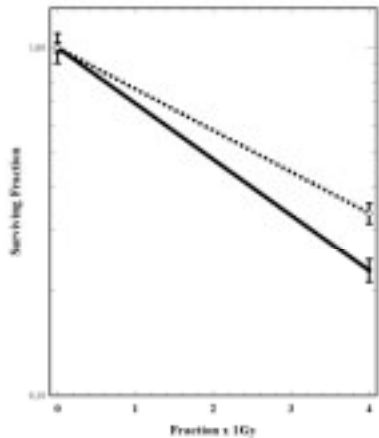


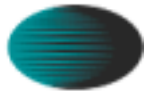
APG4B-siRNA



Control-siRNA

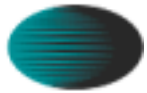
Fractionated
dose irradiation
(4 x 1 Gy)





Summary

- when all details of the role of EGFR and its signalling in the control of DNA-repair under normoxic and hypoxic conditions is understood better and more selective targeting strategies may be developed
- antagonistic strategies to radiation-induced autophagy offer the potential for radiosensitization



Thanks to the current coworkers

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Tim Schickfluß, Techn.

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Christine Rothmund, PhD-Stud.
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Michael Baumann, Dresden

Ekkehard Dikomey, Hamburg