Unità di Radioterapia
Università degli Studi di Brescia

Istituto del Radio "O. Alberti"
A.O. Spedali Civili di Brescia

Incontri Bresciani di Radioterapia Oncologica – Edizione 2013

Brescia Meetings in Radiation Oncology – 2013 Edition

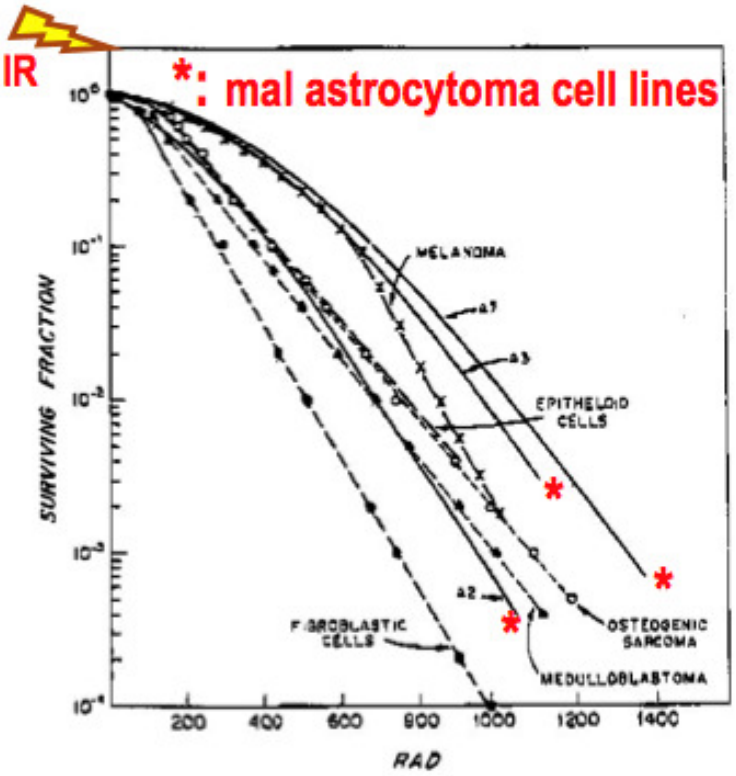
DIFFICULT CLIMBING: TREATMENT OF GLIOMAS AND A TRIBUTE TO PROF. G.P.BITI

Radiobiology of brain tumors: new hints

L. Pirtoli

- ***Molecular Radiobiology of Glioblastoma***
- ***Autophagy in Molecular Radiobiology of Glioblastoma***

Brescia – October 3rd/4th, 2013

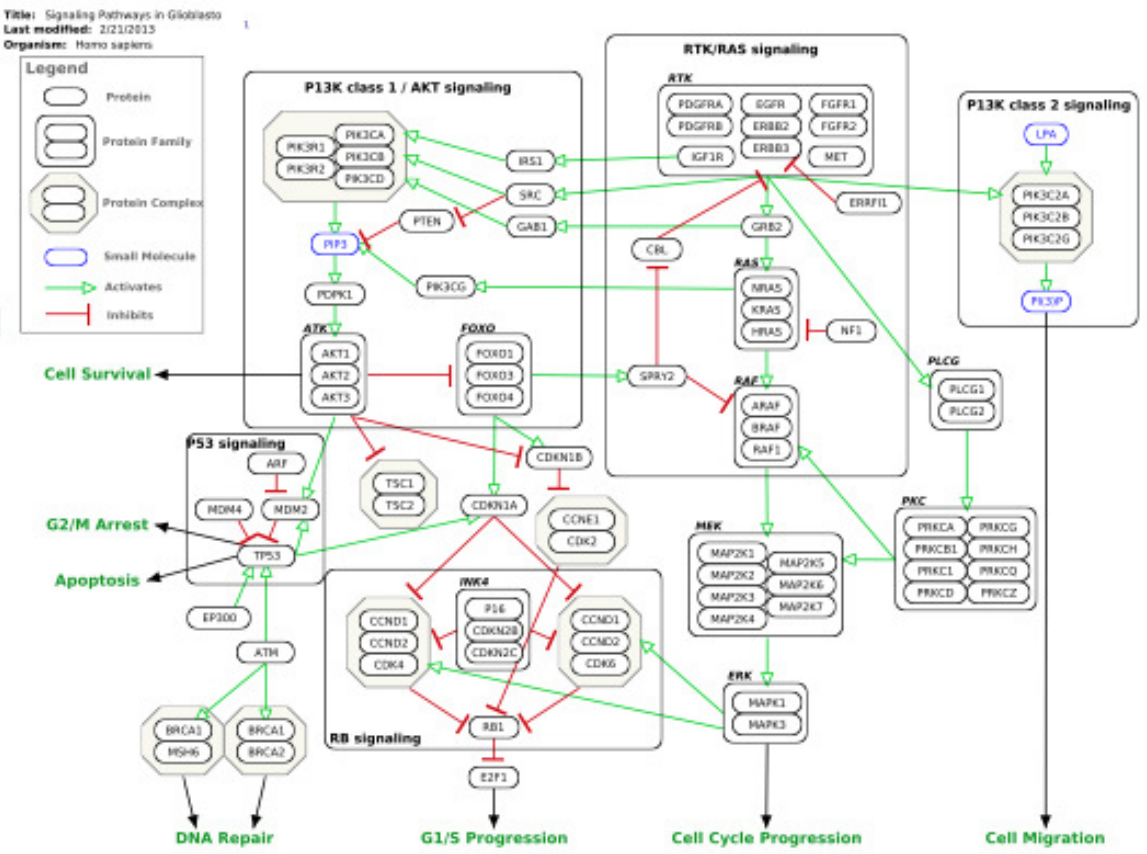


Gerweck LE et al., Radiation Sensitivity of Cultured Glioblastoma Cells. Radiology 1977; 125:231

35 years:
... from math modeling ...

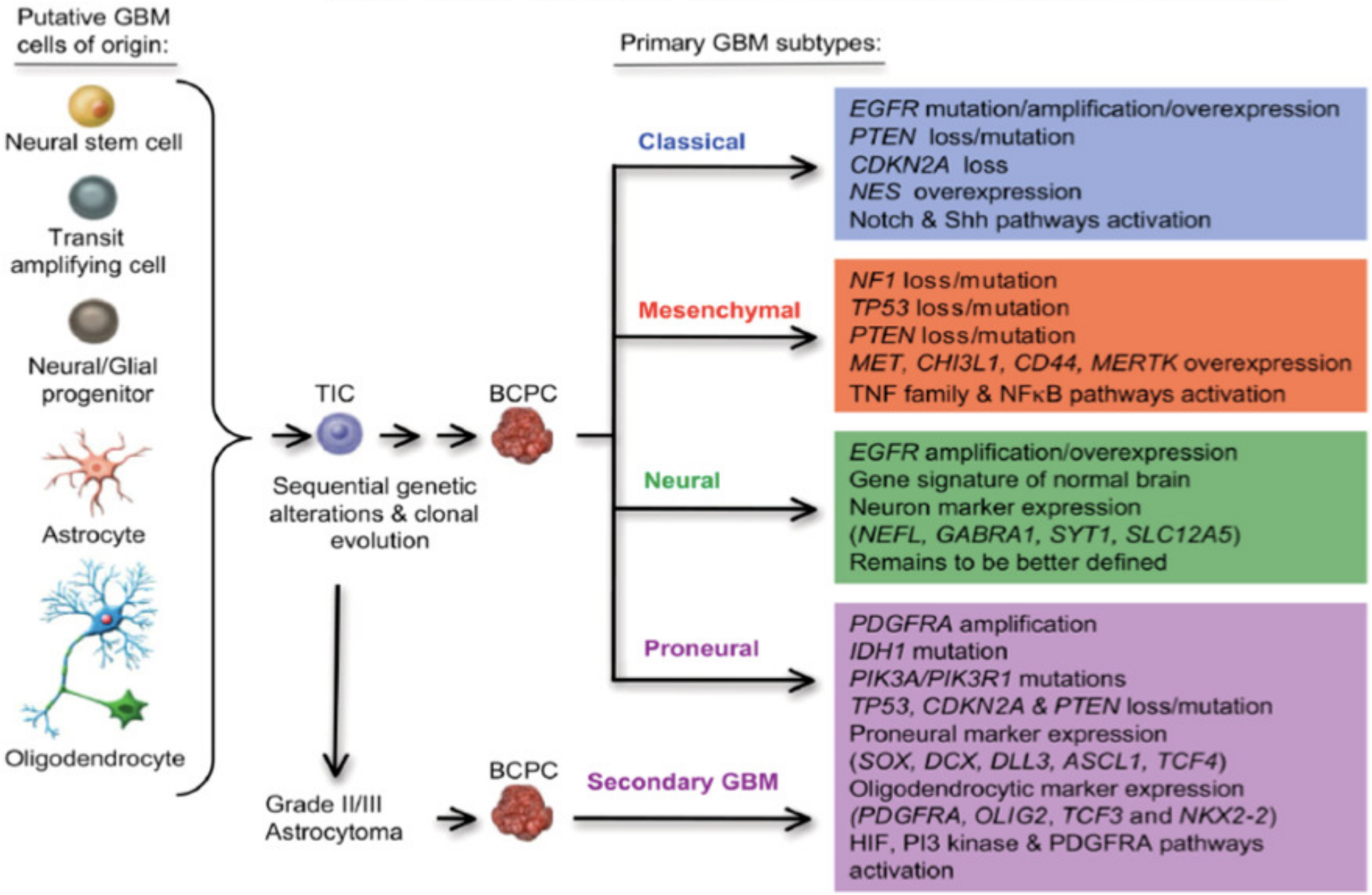
... to mechanistic investigation ...

IR ?

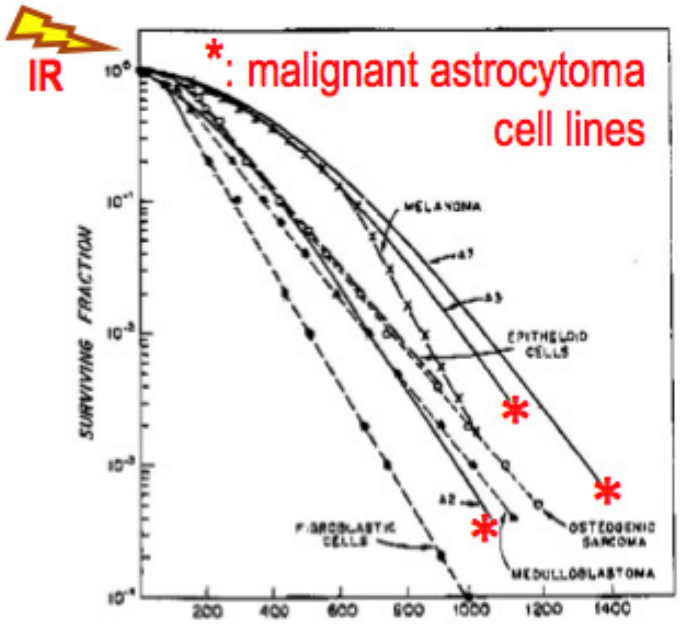


Pico A, Digles D, WikiPathways 2013

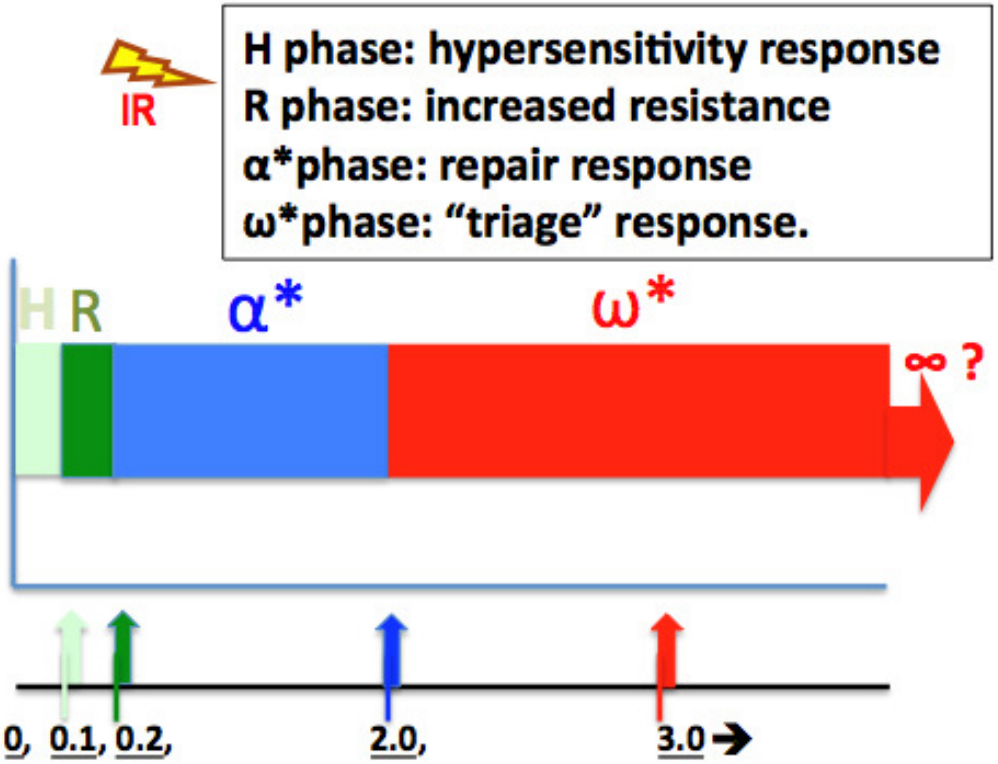
… thus paralleling the improved knowledge in Pathobiology:
THE GENETIC & MOLECULAR CLASSIFICATION FOR GLIOBLASTOMA



Gerweck LE et al., Radiation Sensitivity of Cultured Glioblastoma Cells, Radiology 1977; 125:231

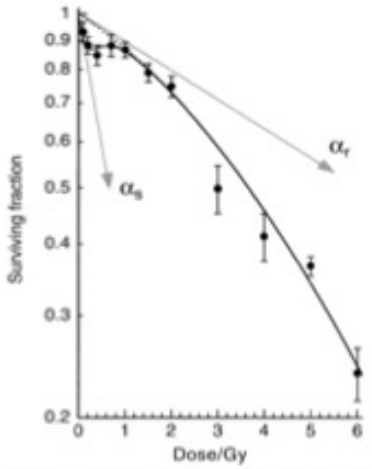


Williams JR, Gridley DS, Slater JB, Radiobiology of Resistant Glioblastoma Cells, www.intechopen 2011; 3-22



35 years:
… from LQ math modeling …

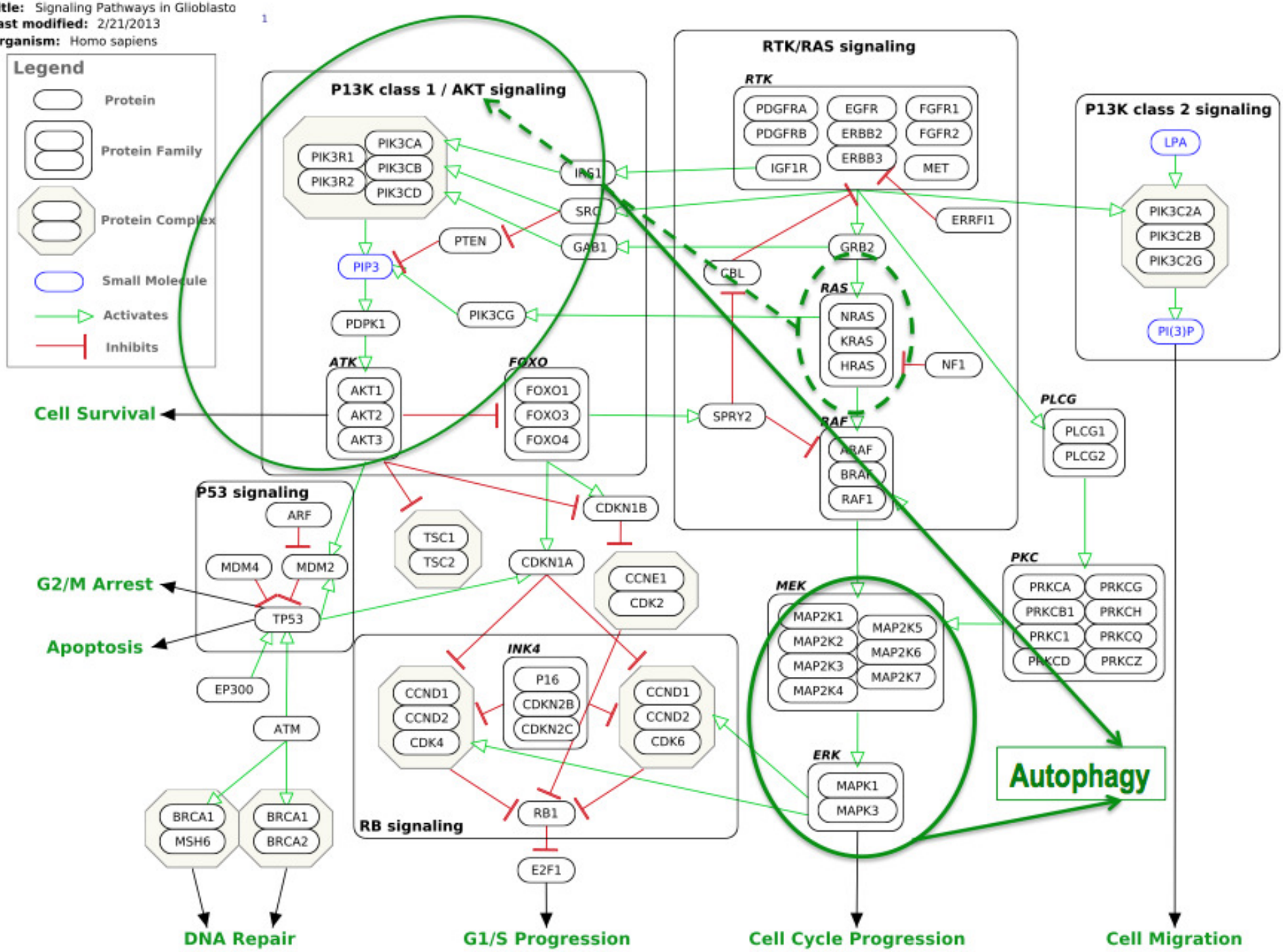
… to inductive approaches to molecular heterogeneity…

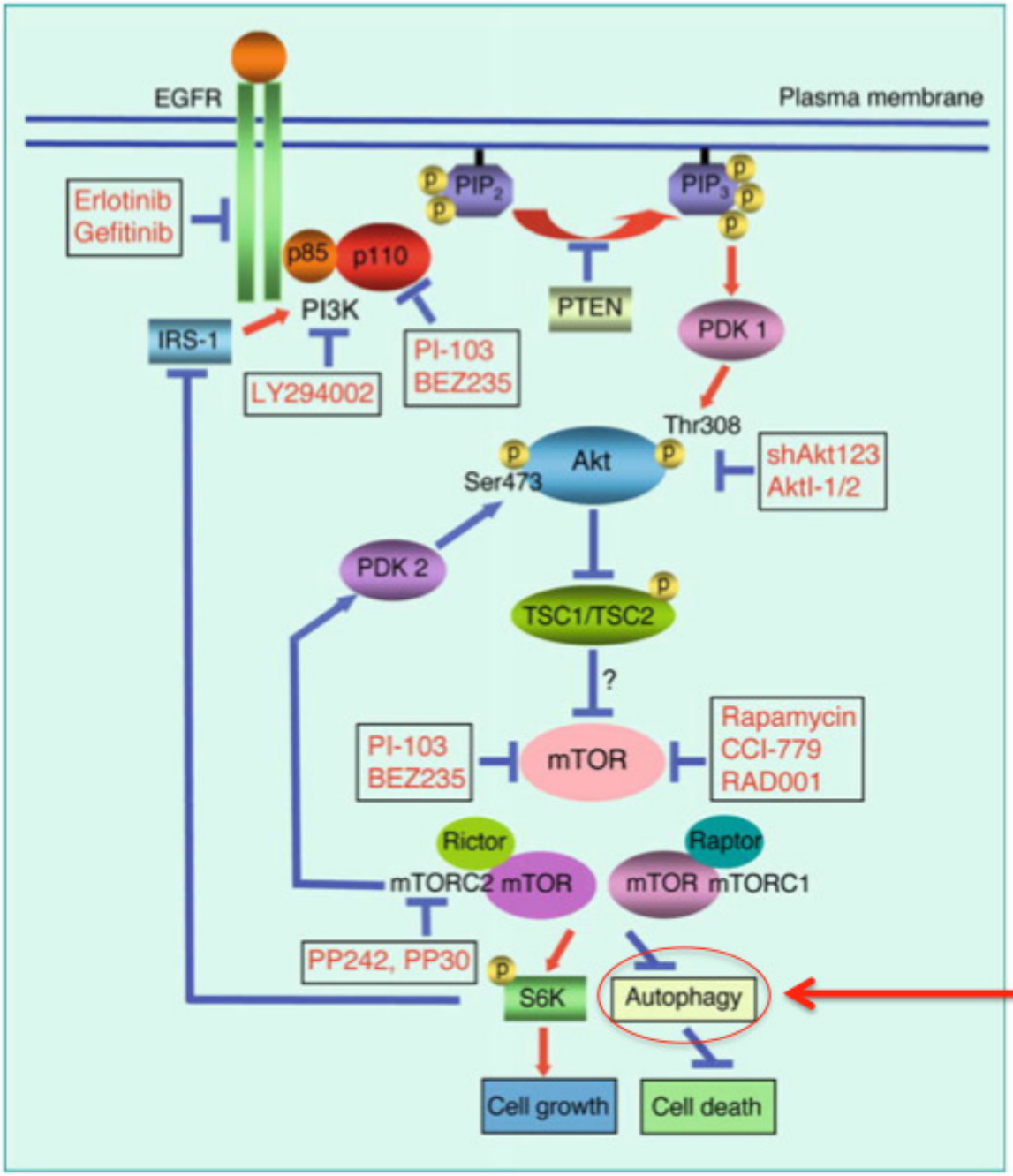


… to mechanistic-based math modeling investigation …

Joiner MC et al., Low-dose hypersensitivity: current status and possible mechanisms. IJROBP 2001; 49:379

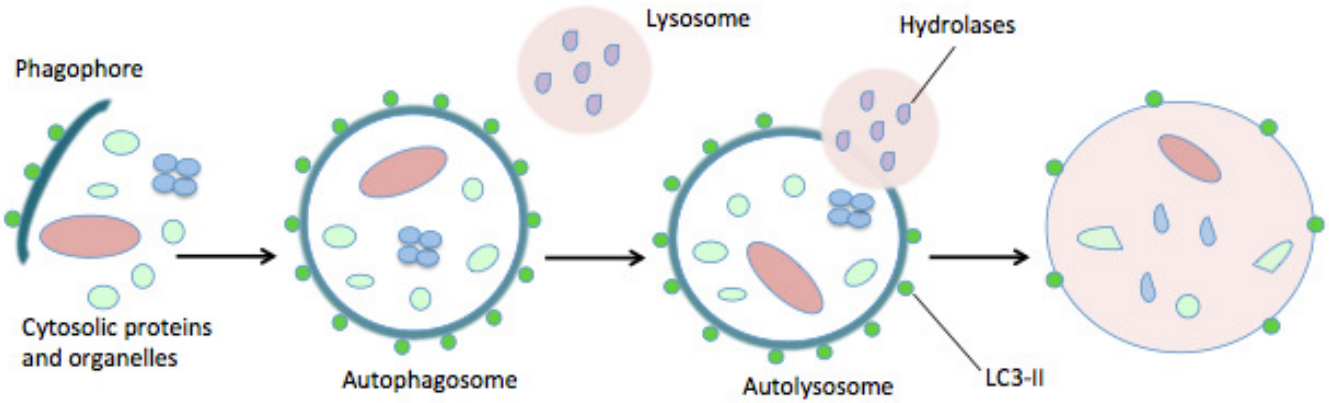
Title: Signaling Pathways in Glioblasto
Last modified: 2/21/2013
Organism: Homo sapiens



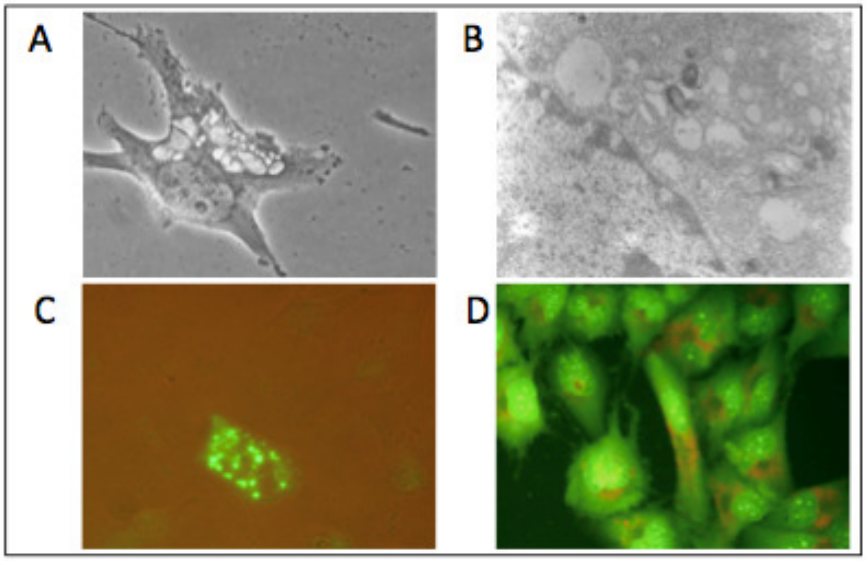


Fan QW, Weiss WA:
Targeting the RTK-PI3K-mTOR Axis in Malignant Glioma: Overcoming Resistance-
Curr Top Microbiol Immunol. 2010; 347: 279–296.

Personal Contributions: Modulating Autophagy as a IR Enhancer in a Cell-Death pathway.



Palumbo S, Comincini S.
J Cell Phys 2013;228:1-8



Autophagy is initiated by the generation of the phagophore, an isolation membrane that likewise derives from the endoplasmic reticulum. This phagophore surrounds the material destined to degradation, and eventually forms a double-membrane vesicle known as autophagosome. Autophagosomes mature by fusing with lysosomes or late endosomes and hence generate auto(phago)lysosomes. Finally, the luminal content of the auto(phago)lysosome is catabolized by acidic hydrolases, resulting in the generation of metabolic substrates that are reexported into the cytosol via permeases of the auto(phago)lysosomal membrane. A Ligh microscopy at 20X resolution (A), electron microscopy (B), LC3B-GFP transduction (C) and orange-acridine staining (D) of human glioma T98G cells after autophagy induction.

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journal homepage: www.thegreenjournal.com

Editorial

Molecular and translational radiation biology/oncology: What's up?

H. Peter Rodemann [a], Bradly G. Wouters [b,c,*]

[a] Department of Radiation Oncology, Eberhard-Karls University of Tübingen, Germany; [b] Ontario Cancer Institute and Campbell Family Institute for Cancer Research, University Health Network, Toronto, Canada; [c] Departments of Radiation Oncology and Medical Biophysics, University of Toronto, Canada

Molecular Radiobiology: the biennial

***International Wofsberg Meetings on Molecular Radiation Biology / Oncology* 1998 - 2013**

Our understanding of the biological contributions to radiation response, and their underlying molecular basis, has seen remarkable progress in recent years. Research areas with the strongest impact on developing new biology driven treatment strategies in radiotherapy include:

- DNA-damage response and repair mechanisms.
- Radiation-induced inter- and intracellular communication and signaling.
- Micro-environmental factors and biological/molecular imaging.
- Tumor profiling, biomarkers and molecular targeting.

The four "Wolfsberg's domains of Molecular Radiation Biology"

The four "Wolfsberg's domains of Molecular Radiation Biology"

- DNA-damage response and repair mechanisms.
- Radiation-induced inter- and intracellular communication and signaling.
- Micro-environmental factors and biological/molecular imaging.
- Tumor profiling, biomarkers and molecular targeting.

All of these domains are involved by AUTOPHAGY.
Janji B et Al, 2013: *Role of Autophagy in Cancer and Tumor Progression*. Chapter 9, INTECH Open Science,

AUTOPHAGY

- Autophagy: a phylogenetic-preserved mechanism devoted to degrade long-lived proteins, and cytoplasmic organelles. A membranous organelle is involved (autophagosome), that is, a double-membrane vescicle that progressively engulf cytoplasmic constituents and delivers them to lysomes for degradation.
- It may act as a **pro-survival mechanism** to several kinds of stresses (e.g.: damaged mitochondria, protein aggregation, pathogens, starvation).
- It may also act as a **pro-death mechanism** (the so-called Type II programmed cell death [PCD], or autophagy-associated PCD), morphologically and biochemically different from apoptosis (Type I PCD).
- Studies on apoptotic-defective cells suggest that autophagic PCD might emerge as a cell death mechanism once the primary PCD pathway is inhibited. Autophagy PCD is activated in cells derived from breast, colon, prostate and brain cancers, in response to anti-cancer drugs and to Ionizing Radiation (IR).

Autophagy may exert a pro-survival effect, as suggested by autophagy inhibition by silencing some autophagic genes, resulting in enhancement of IR sensitivity.

Cancer Research

AACR

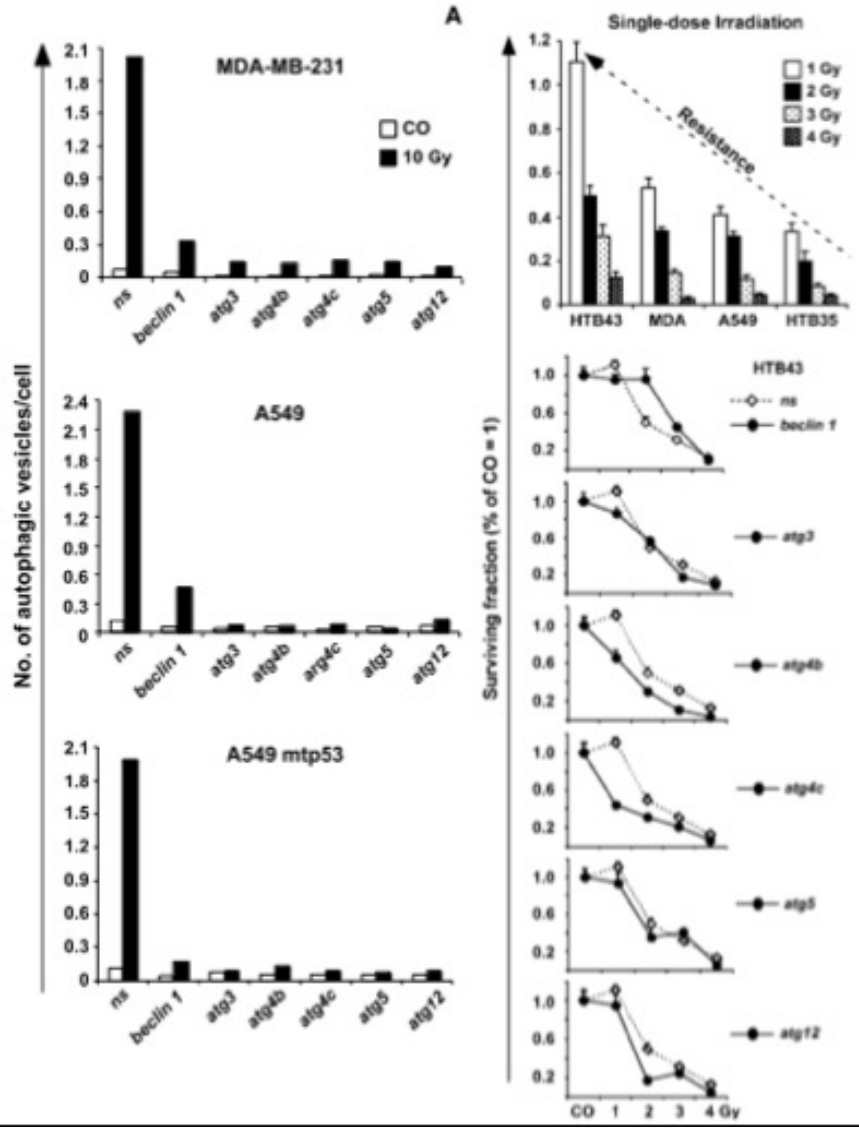
Blocked Autophagy Sensitizes Resistant Carcinoma Cells to Radiation Therapy

Anja Apel, Ingrid Herr, Heinz Schwarz, et al.

Cancer Res 2008;68:1485-1494. Published online March 3, 2008.

... In conclusion, inhibition of autophagy may sensitize cancer cells to radiation, whereas basal clonogenicity of untreated resistant cells may be even enhanced by inhibition of autophagy. Our data suggest that inhibition of autophagy in cancer cells may vary dependent on the type of cancer, individual characteristics of cancer cells, microenvironments, and therapeutic treatment. In our system, short time inhibition of autophagy was beneficial to enhance cytotoxicity of radiotherapy in resistant cancer cells.

Note: GB cell lines were not studied in this experience.



Autophagy may exert a pro-survival effect, as suggested by autophagy inhibition by silencing (shRNA) some autophagic genes, resulting in enhancement of IR sensitivity in a primary GB CD133⁺ cell line.

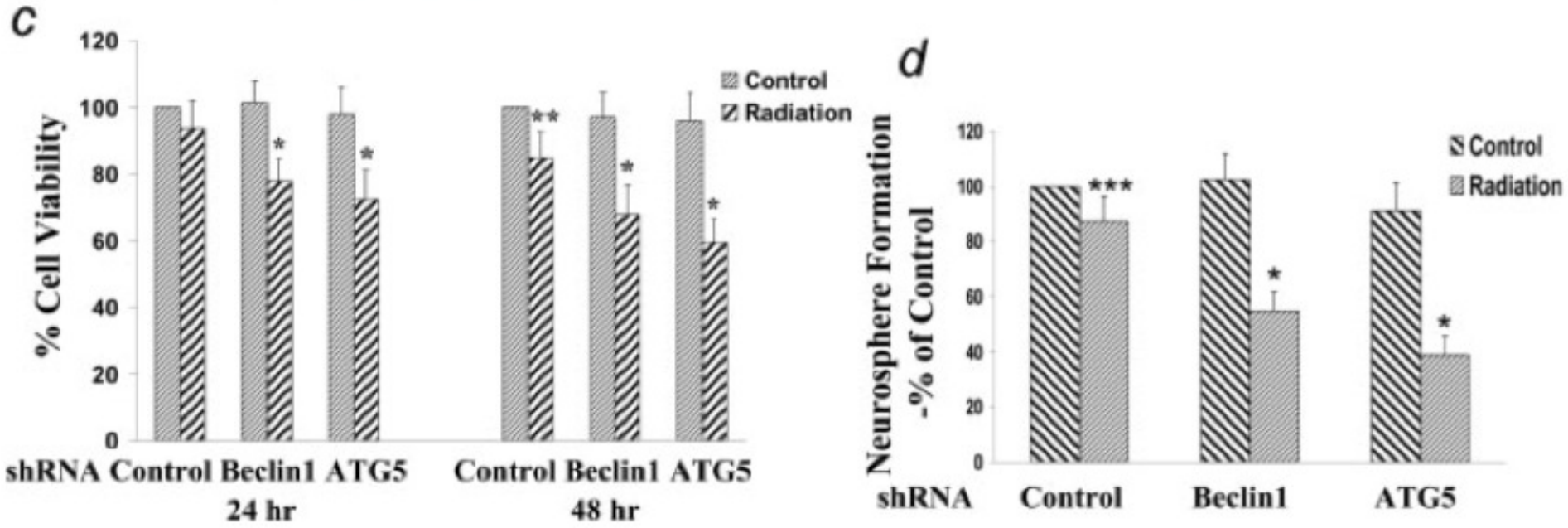
Int. J. Cancer: **125,** 717–722 (2009)

SHORT REPORT

The induction of autophagy by γ-radiation contributes to the radioresistance of glioma stem cells

Stephanie L. Lomonaco[1], Susan Finniss[1], Cunli Xiang[1], Ana DeCarvalho[1], Felix Umansky[1], Steven N. Kalkanis[1], Tom Mikkelsen[1] and Chaya Brodie[1,2]*

[1]*William and Karen Davidson Laboratory of Cell Signaling and Tumorigenesis, Department of Neurosurgery, Hermelin Brain Tumor Center, Henry Ford Hospital, Detroit, MI*
[2]*Mina and Everard Goodman Faculty of Life-Sciences, Bar-Ilan University, Ramat-Gan, Israel*



Protein and mRNA expression of autophagy gene Beclin 1 in human brain tumours.

Miracco C, Cosci E, Oliveri G, Luzi P, Pacenti L, Monciatti I, Mannucci S, De Nisi MC, Toscano M, Malagnino V, Falzarano SM, **Pirtoli L**, Tosi P.

Int J Oncol. 2007 Feb;30(2):429-36.

"... in most high-grade astrocytic, ependymal neoplasms and atypical meningiomas we found a decrease of cytoplasmic protein expression that was, instead, high in the majority of low-grade tumours ... . The expression level of Beclin 1 mRNA was significantly lower in glioblastomas than in grade II (p=0.04) and grade I (p=0.01) astrocytomas ... ."

The prognostic role of Beclin 1 protein expression in high-grade gliomas.

Pirtoli L, Cevenini G, Tini P, Vannini M, Oliveri G, Marsili S, Mourmouras V, Rubino G, Miracco C.

Autophagy. 2009 Oct;5(7):930-6. Epub 2009 Oct 8.

The protein expression level of Beclin 1 was significantly related to prognosis in HGGs

Table 2 **Survival results and monovariate analysis (log-rank test)**

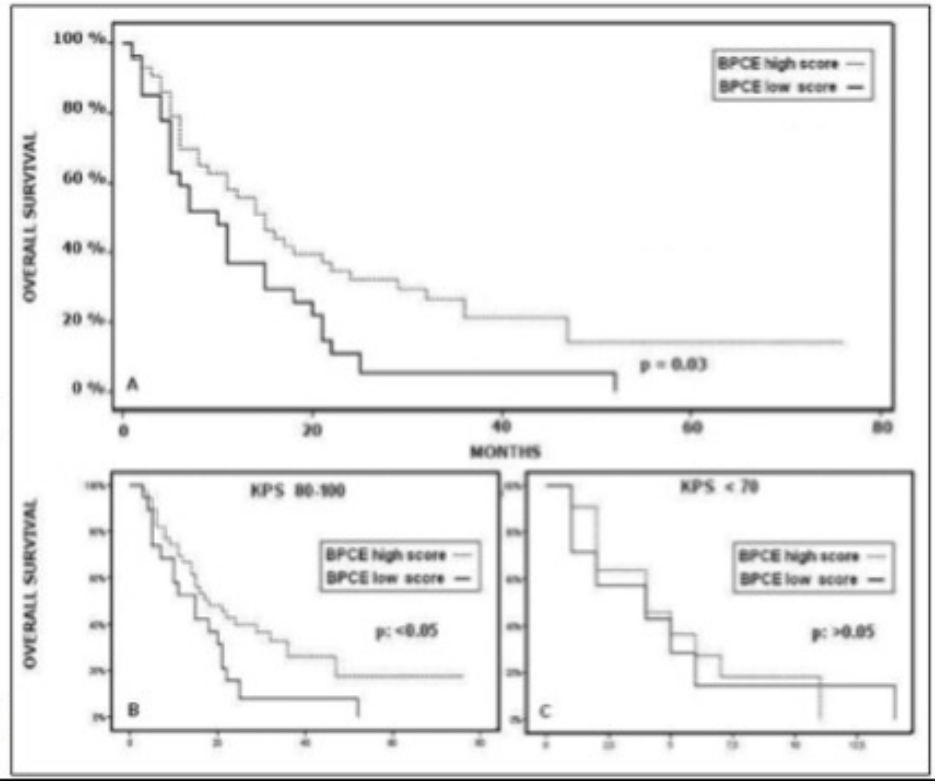
		Median survival (months)	1 y	2 y	p
Whole series		12 m	50%	25%	
Age	≤50 y	18 m	70%	47%	0.035
	≥50 y	11 m	42%	18%	
Grading	AA	29 m	71%	71%	0.014
	GB	11 m	46%	19%	
KPS	80–100%	17 m	62%	32%	0.000
	≤70%	4 m	6%	0%	
BPCE	High score 47 (61.75%)	15 m	56%	32%	0.030
	Low score 29 (38.25%)	10 m	37%	11%	
Surgery	Gross total resection	25 m	85%	55%	0.000
	Partial resection/Biopsy	8 m	36%	13%	
RT	Radical, PBI, D = 60–70 Gy	16 m	64%	34%	0.000
	Palliative, WBI, D ≤50 Gy	4 m	19%	5%	
TMZ CHT	Yes	17 m	64%	32%	0.001
	No	5 m	27%	15%	
Treatment	Optimal	20 m	73%	39%	0.030
	Sub-optimal	6 m	30%	13%	
MGMT	Unmethylated	4 m	13%	8%	0.000
	Methylated	25 m	72%	51%	

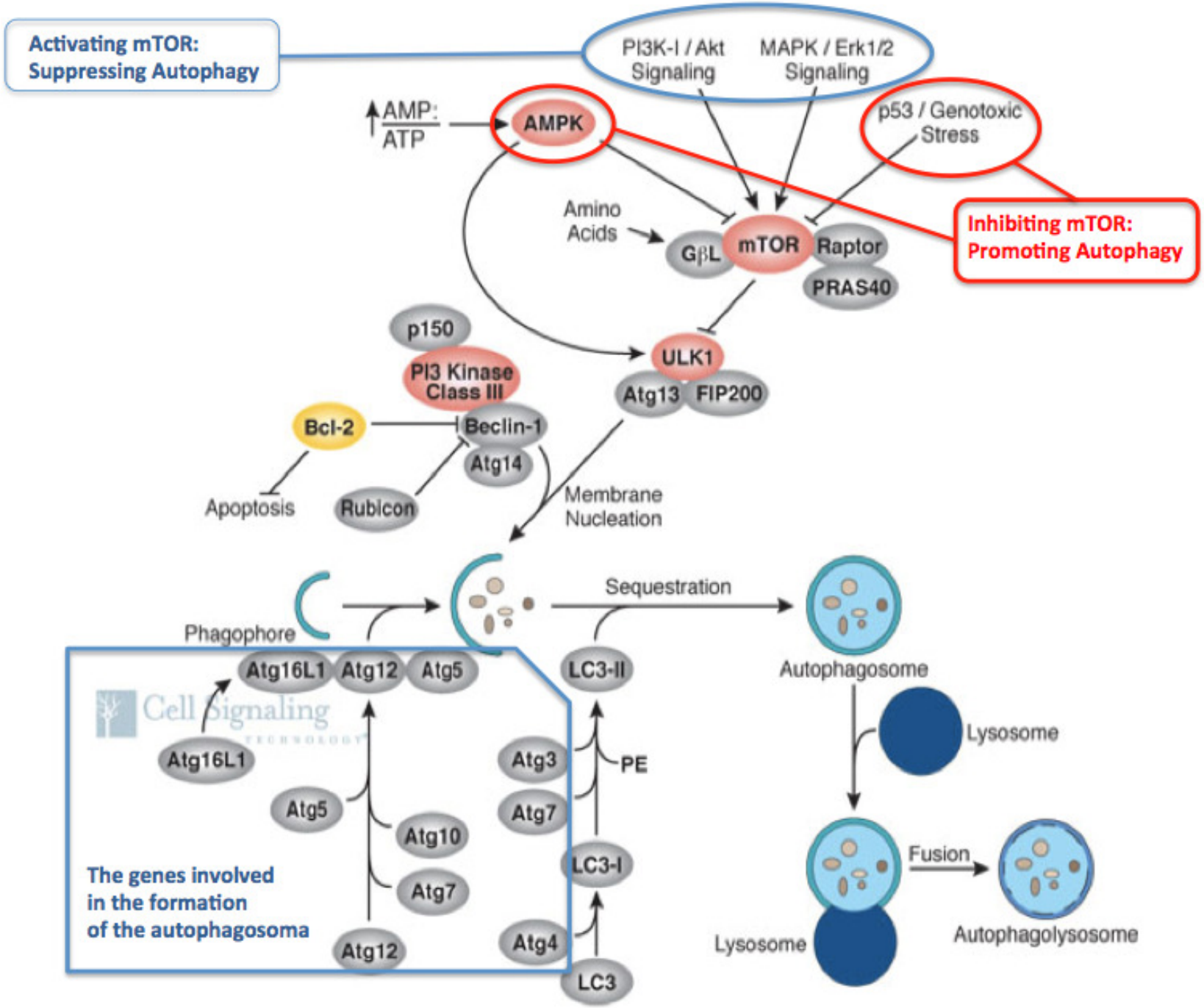
MGMT methylation status was analyzed in 52 out of 76 patients. AA = Anaplastic Astrocytoma; GB = Glioblastoma; KPS = Karnofski Performance Status; BPCE = Beclin1 Protein Cytoplasmic Expression; RT = Radiotherapy; PBI, = Partial Brain Irradiation; WBI = Whole Brain Irradiation; TMZ = Temozolomide; CHT = Chemotherapy; MGMT = Gene Promoter Methylation Status.

Table 3 **Multivariate analysis (Cox regression test)**

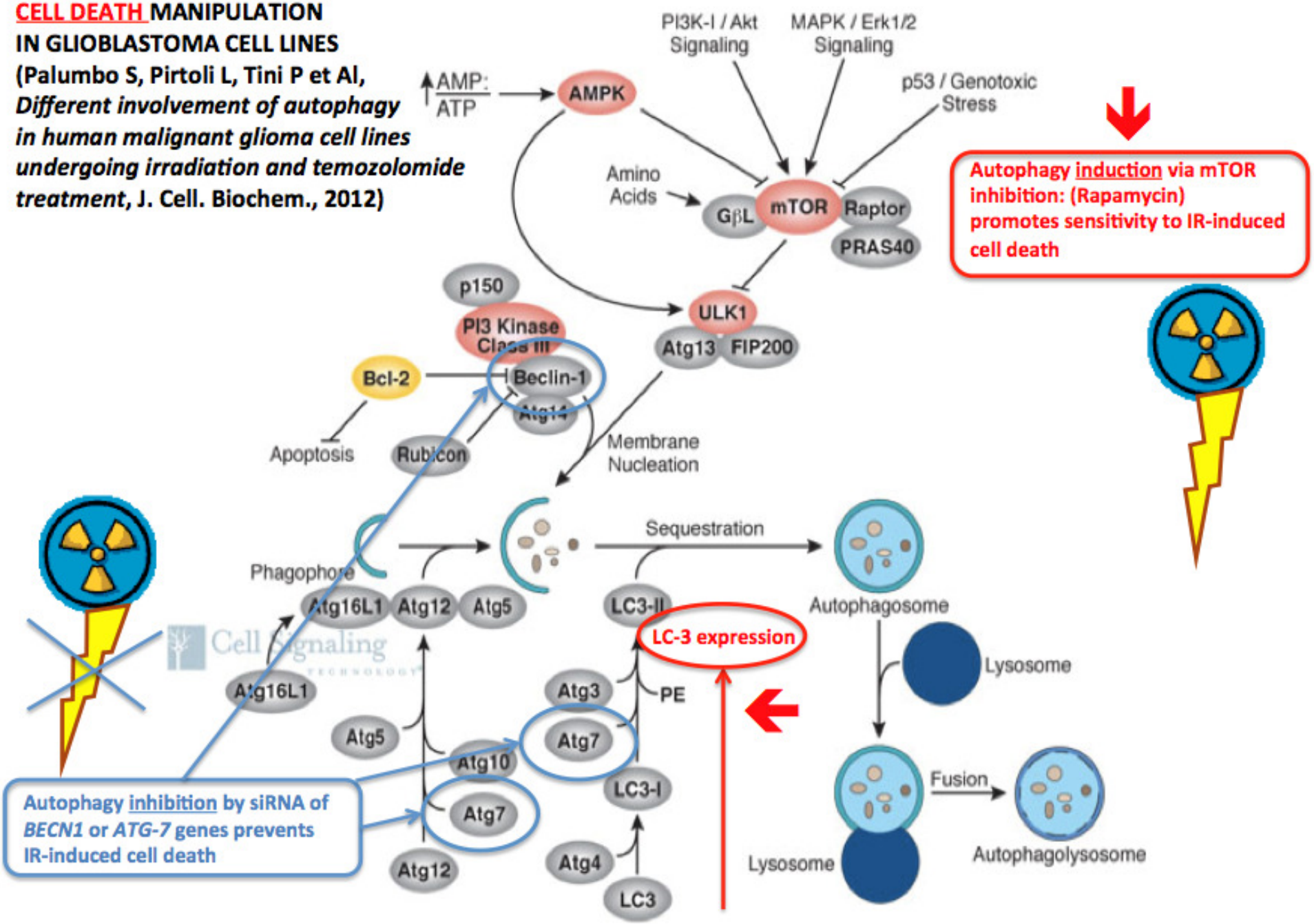
	OR	p	CI (95%)
Age >50	1.97	0.044	1.02–3.81
Grading: GB	3.86	0.025	1.19–12.59
KPS ≤70%	6.10	0.000	3.22–11.55
BPCE: Low score	1.74	0.037	1.03–2.94
Surgery: Partial resection/Biopsy	3.48	0.000	1.78–6.81
RT: Palliative, WBI, D ≤50 Gy	3.50	0.000	2.02–5.95
TMZ CHT: No	2.24	0.002	1.36–3.71
Treatment: Sub-optimal	2.57	0.000	1.52–4.33

Odds ratio (OR), p values, and confidence interval (CI) are reported. GB = Glioblastoma; KPS = Karnofski Performance Status; BPCE = Beclin1 Protein Cytoplasmic Expression; RT = Radiotherapy; WBI = Whole Brain Irradiation; TMZ = Temozolomide; CHT = Chemotherapy.




Activating mTOR:
Suppressing Autophagy
PI3K-I / Akt Signaling
MAPK / Erk1/2 Signaling
AMP:
ATP
AMPK
p53 / Genotoxic Stress
Inhibiting mTOR:
Promoting Autophagy
Amino Acids
GβL
mTOR
Raptor
PRAS40
p150
PI3 Kinase Class III
ULK1
Atg13
FIP200
Bcl-2
Beclin-1
Atg14
Apoptosis
Rubicon
Membrane Nucleation
Phagophore
Sequestration
Autophagosome
Atg16L1
Atg12
Atg5
LC3-II
Cell Signaling
TECHNOLOGY
Lysosome
Atg16L1
Atg3
PE
Atg5
Atg7
Atg10
LC3-I
Fusion
The genes involved in the formation of the autophagosoma
Atg7
Atg12
Atg4
Lysosome
Autophagolysosome
LC3

AUTOPHAGY-RELATED
CELL DEATH MANIPULATION
IN GLIOBLASTOMA CELL LINES
(Palumbo S, Pirtoli L, Tini P et Al, ***Different involvement of autophagy in human malignant glioma cell lines undergoing irradiation and temozolomide treatment*****, J. Cell. Biochem., 2012)**



AUTOPHAGY-RELATED CELL DEATH MANIPULATION IN GLIOBLASTOMA CELL LINES
(Palumbo S, Pirtoli L, Tini P et Al, *Different involvement of autophagy in human malignant glioma cell lines undergoing irradiation and temozolomide treatment*, J. Cell. Biochem., 2012)

Cell-death investigation in T98G cells.

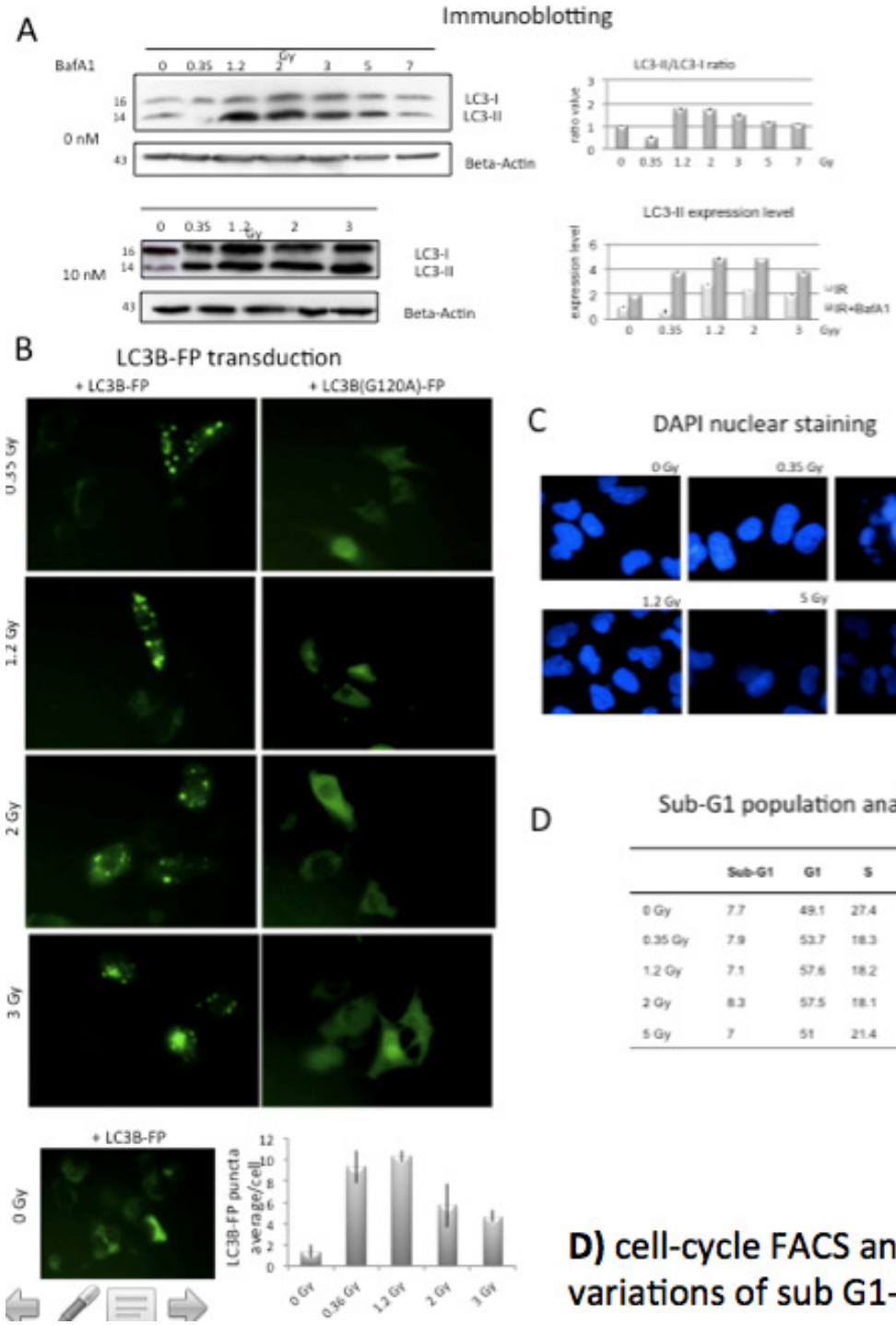
LC3-I to LC3-II conversion, BacMam LC3B-DFB transducion, DAPI staining and sub-G1 apoptotic population analysis.

A) LC3-II/LC3-I ratio: higher in IR than in NC cells;

B) to discern whether increase in LC3-II was due to autophagy or lysosomal accumulation, BafA1 10nM was added ➔ further increase in LC3-II level at each IR dose, compared to NC: autophagic flux enhanced at low-intermediate IR doses (evaluated also by MacMam 2.0 viral vector, transducing and expressing LC3-II fluorescent LC3B protein). NC and transducted only cells: diffuse F. pattern; IR cells: punctate F. pattern (LC3B-GFP accumulation on autophagosome-like vescicles).

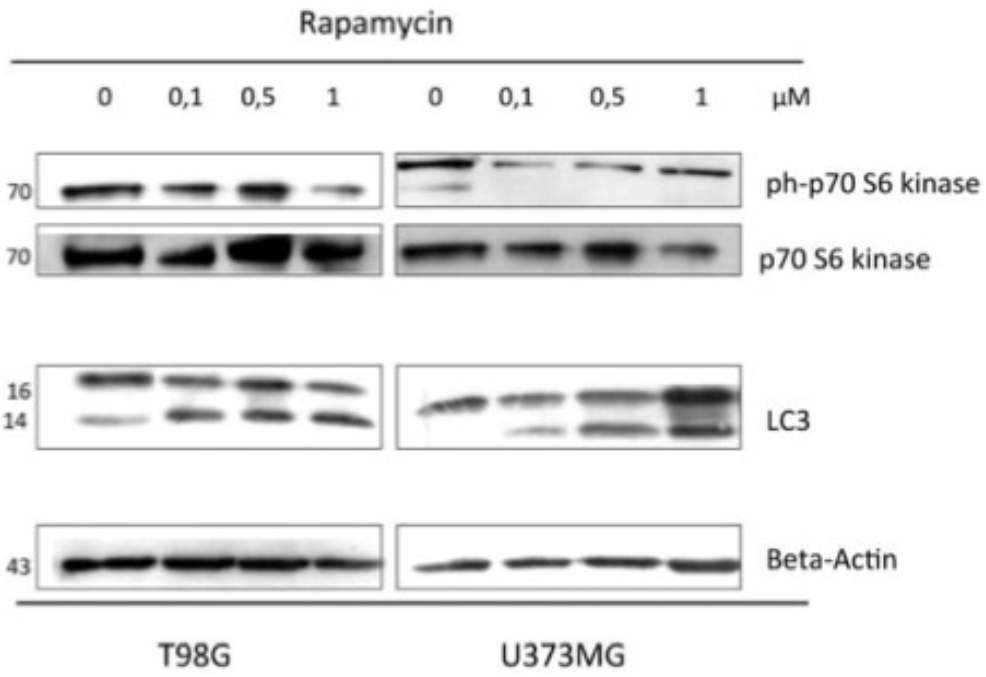
C) DAPI-staining: no apoptotic features (nuclear fragmentation, chromatin condensation, apoptotic bodies), also with Rapamycin. Staurosporine highlighted apoptotic features.

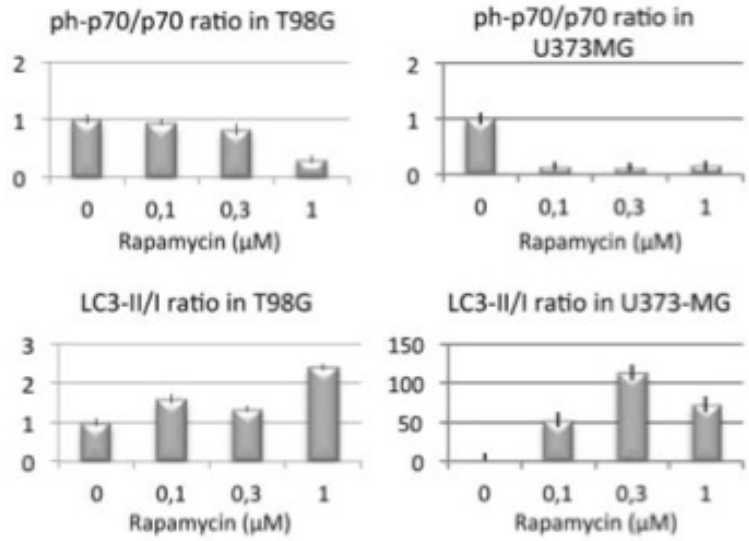
D) cell-cycle FACS analysis: sub-G1 apoptotic population. No appreciable variations of sub G1-pop. between IR cells and NC: no apoptosis induced.



	Sub-G1	G1	S	g2/M
0 Gy	7.7	49.1	27.4	21.5
0.35 Gy	7.9	53.7	18.3	18.8
1.2 Gy	7.1	57.6	18.2	15.9
2 Gy	8.3	57.5	18.1	15.3
5 Gy	7	51	21.4	21.6

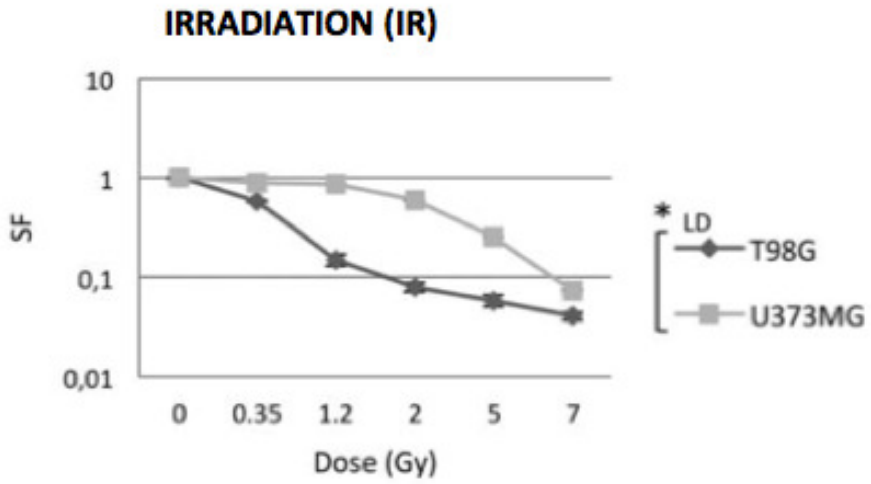
Palumbo S, Pirtoli L, Tini P et Al,
***Different involvement of autophagy in human malignant glioma cell lines undergoing irradiation and temozolomide treatment*, J. Cell. Biochem., 2012**

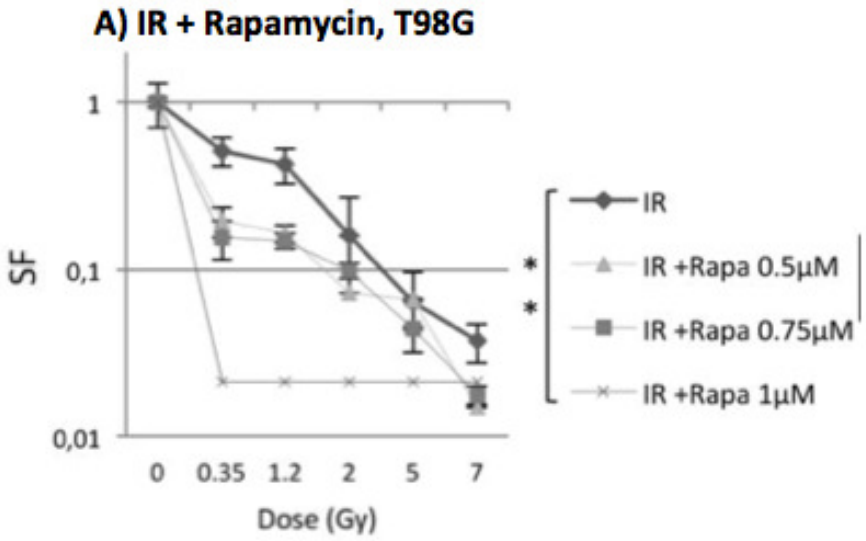


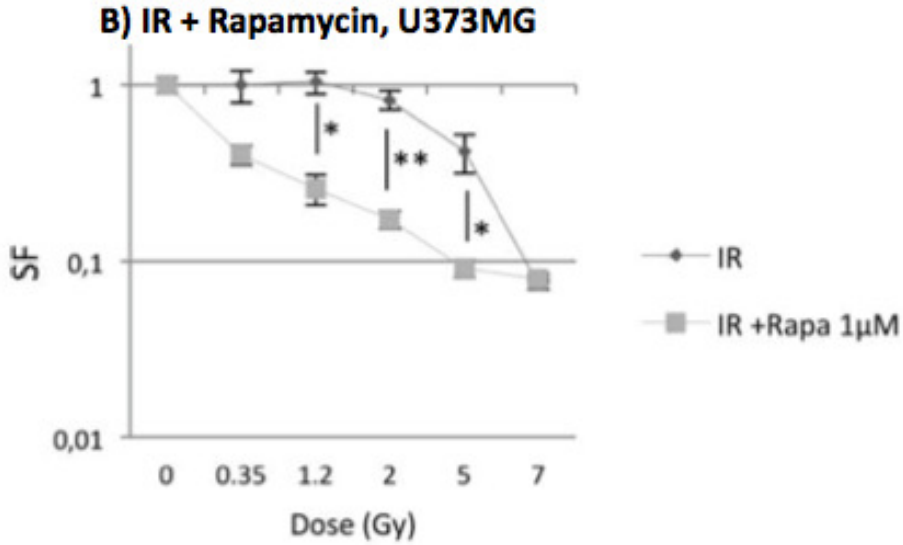


Autophagy induction by Rapamycin in T98G and U373MG GB cell lines:
Evaluation of ph-p70S6K, p70S6K, and LC3 expression by immunoblotting, after 24h rapamycin incubation (0.1, 0.5, 1 µM)
Note: Protein expression is normalized with Beta-Actin for densitometric analysis and referred to the expression of untreated cells.

Palumbo S, Pirtoli L, Tini P et Al,
***Different involvement of autophagy in human malignant glioma cell lines undergoing irradiation and temozolomide treatment*, J. Cell. Biochem., 2012**



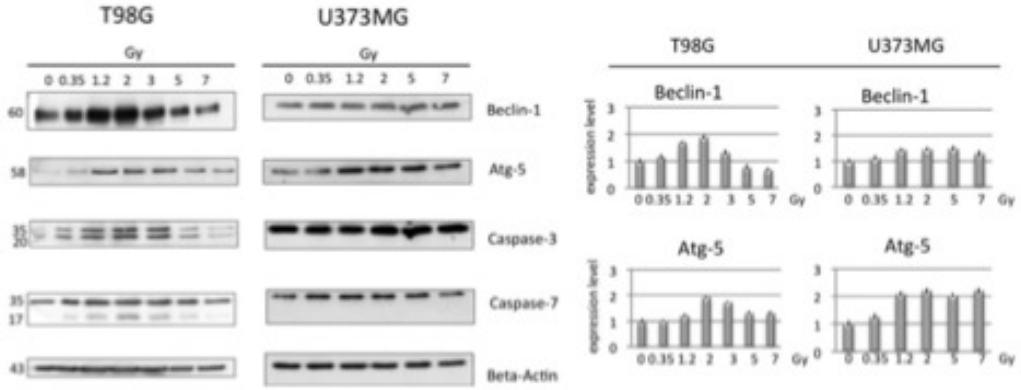




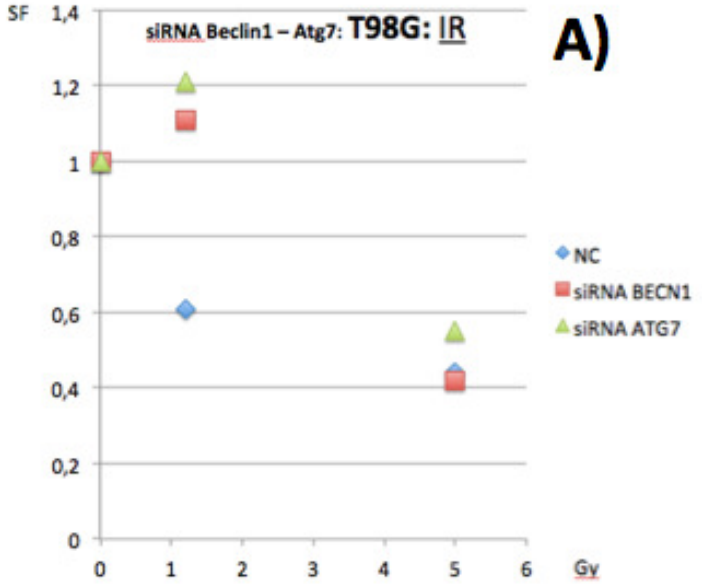
Autophagy induction by Rapamycin:

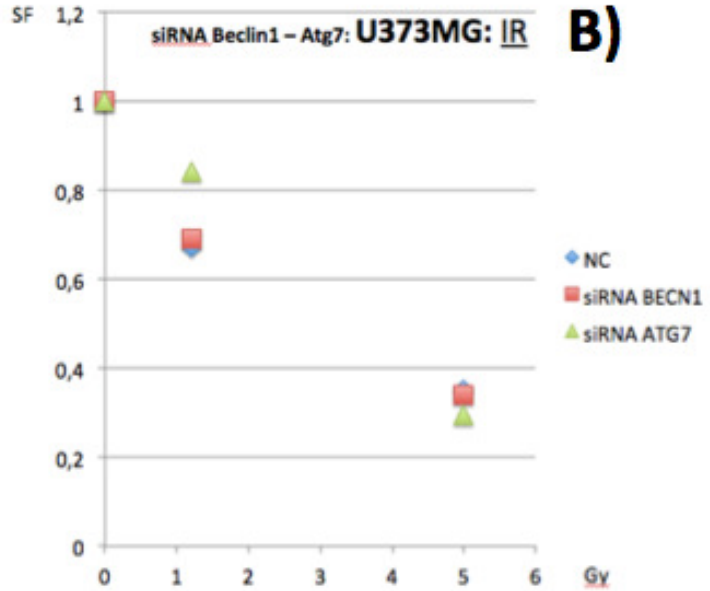
- an enhancement of the effect of IR in T98G is observed with increasing concentrations, and it is more marked at low doses;
- a significant enhancement of the effect of IR is observed also in U373MG, except for the highest dose.

Palumbo S, Pirtoli L, Tini P et Al,
***Different involvement of autophagy in human malignant glioma cell lines undergoing irradiation and temozolomide treatment*, J. Cell. Biochem., 2012**



Cell-death pathways involved in IR-sensitivity: :
T98G: protein expression: Becn1 and Atg5 (autophagy markers) highly expressed at low-intermediate doses, and a weak Caspase-3/7 cleavage (just early apoptosis activation);
U373MG: protein expression: Becn1 (poorly) and Atg5 expressed only at intermediate-high doses; no Caspase-3/7 cleavage (no apoptosis activation);





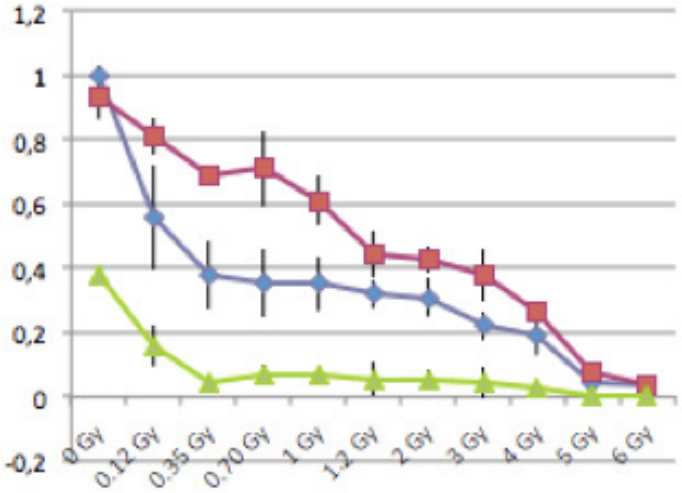
Autophagy inhibition by knocking-down *Becn1* and *Atg7* through siRNA transfection:

- A) autophagy inhibition totally prevented 1.2 Gy IR effect on SF of T98G;
- B) no relevant effect of autophagy inhibition was shown in U373MG.

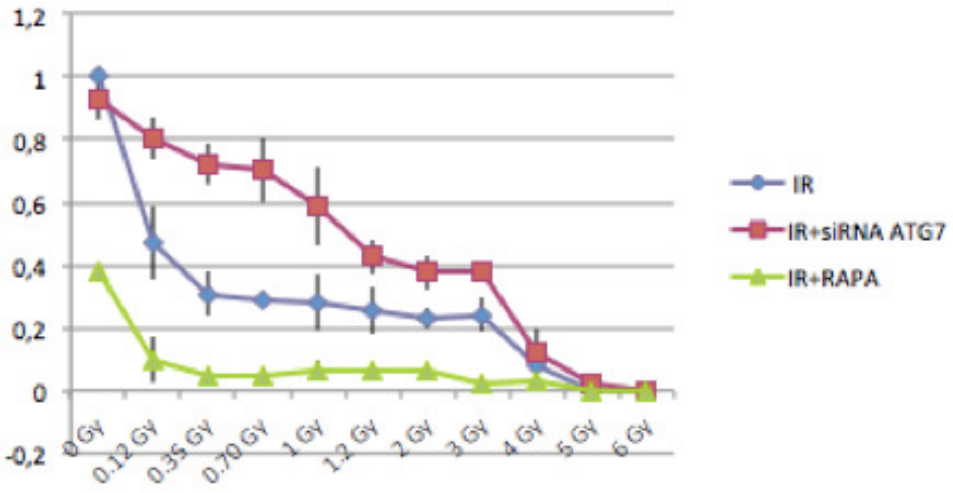
Autophagy induction in T98G and U373MG enhances IR cell-killing mostly at low-doses. This effect occurs also at low dose-rates. Autophagy suppression reduces IR sensitivity in T98G and has no effect in U373MG (L. Pirtoli, S. Palumbo, P. Tini, 2013: unpublished data).

T98G

240 cGy/min

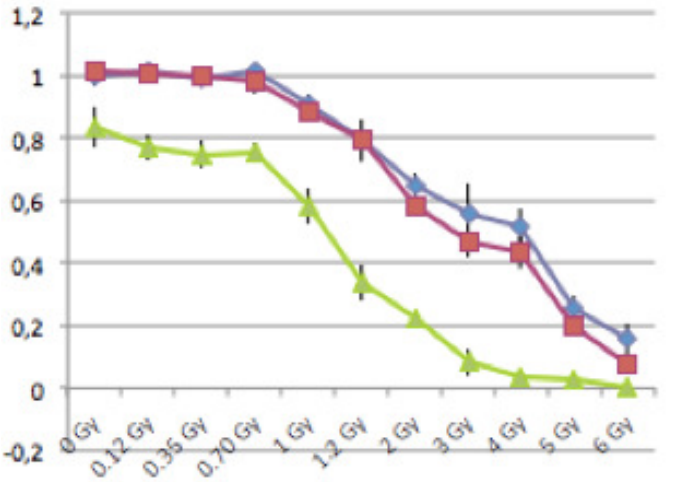


12.5 cGy/min

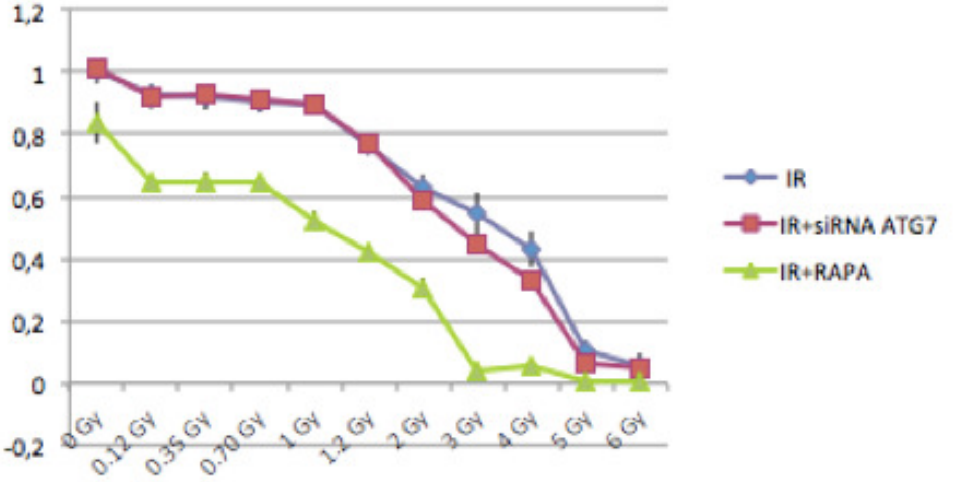


U373MG

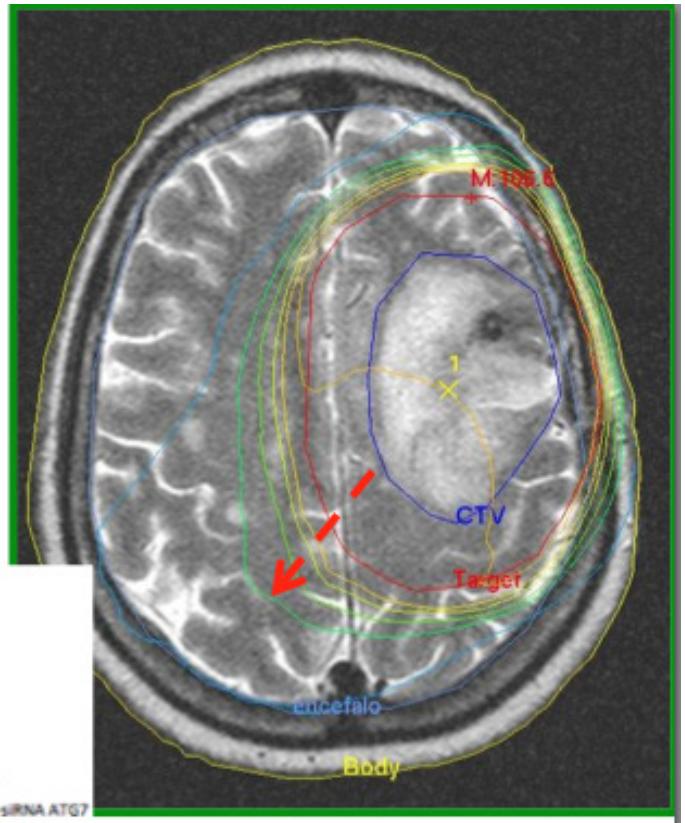
240 cGy/min



12.5 cGy/min



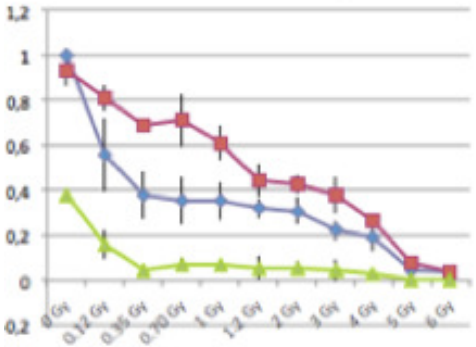
This might have a relevant effect in the gradient region, if reproduced *in vivo*, and could be optimized through a "Gradient Modulation" methodology.



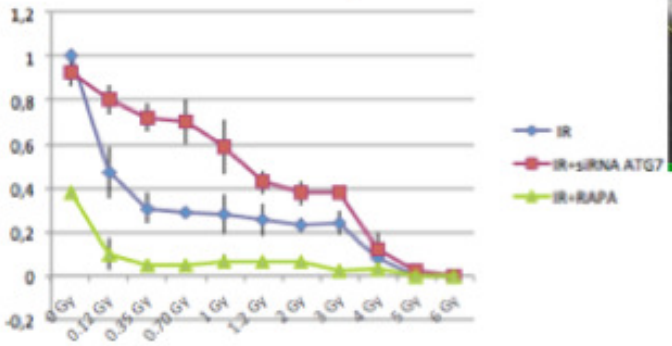
inverse IR enhancement effect with gradient

T98G

240 cGy/min

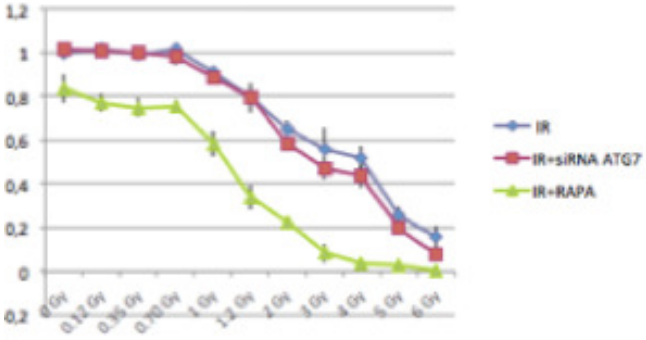


12.5 cGy/min



U373MG

240 cGy/min



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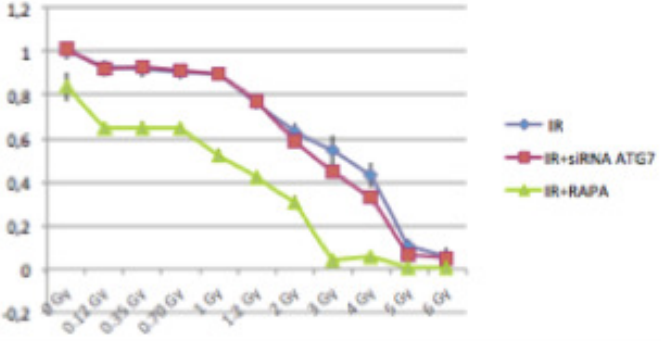
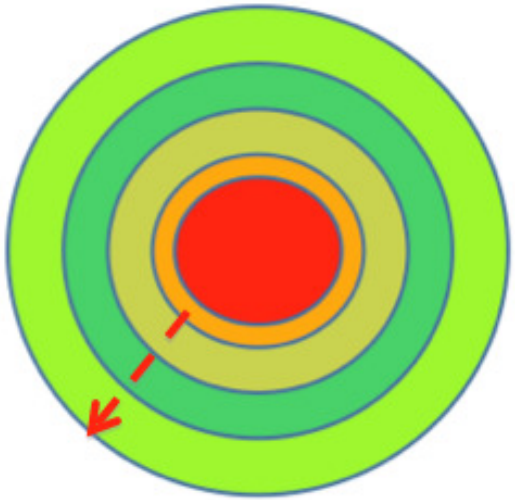




Epidermal Growth Factor Receptor (*EGFR*)
Expression correlates with clinical and pathological features, response to therapy, and survival in Glioblastoma. A preliminary report based on a patient series.
(P. Tini, G. Rubino, S. Palumbo, A. Cerase, L. Pirtoli, C. Miracco, 2013, unpublished data).

68 pts, 2007➔ 2011;

IHC EGFR -/+: 23/68;
EGFR ++/+++: 45/68

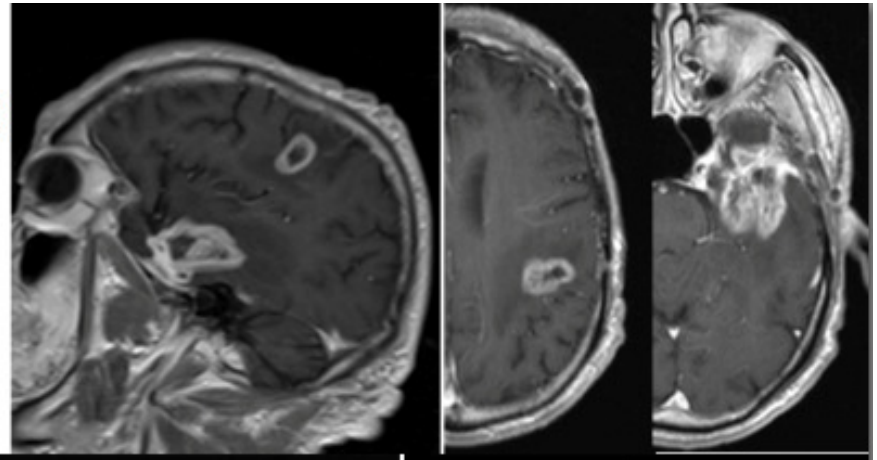
RATE OF RE-GROWTH:
(mean, after RT+TMZ)

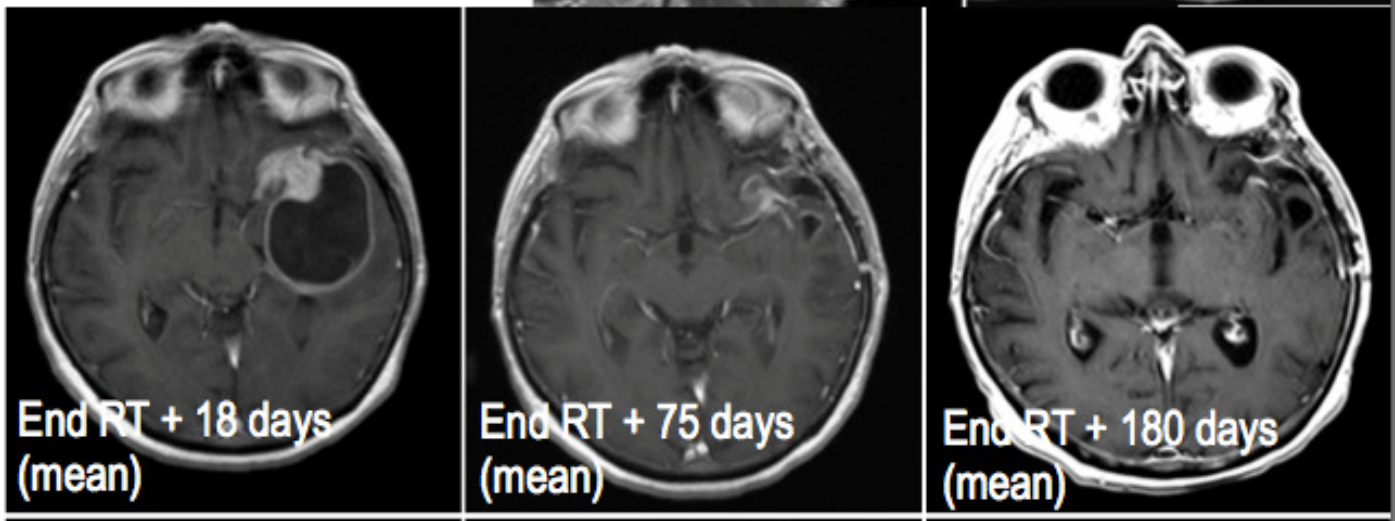
EGFR-/+: - 69.5%

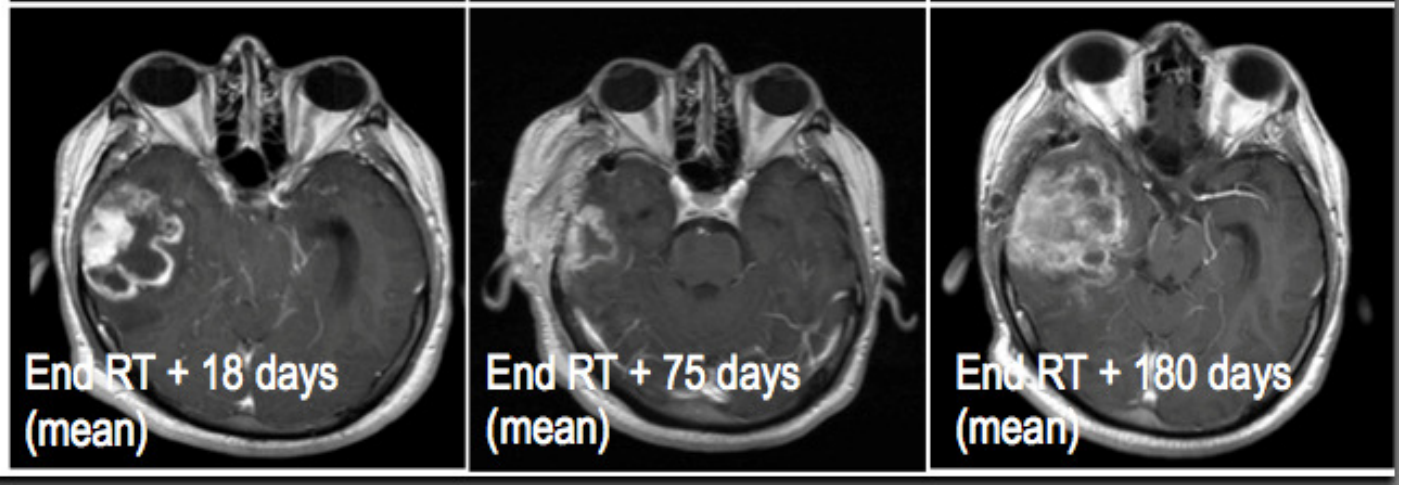
EGFR++/+++: + 139.5%

p= 0.002

MULTIFOCALITY
(SYNCR., METACR.):
EGFR-/+: 0/23
EGFR++/+++: 20/45
Syncr. p= .001
Metacr. p= .002







Epidermal Growth Factor Receptor (*EGFR*) Expression correlates with clinical and pathological features, response to therapy, and survival in Glioblastoma. A preliminary report based on a patient series.
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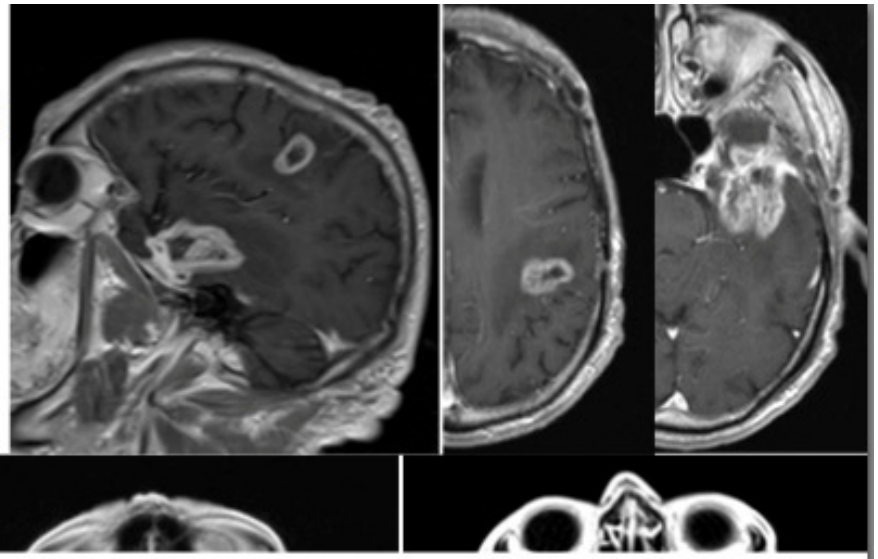

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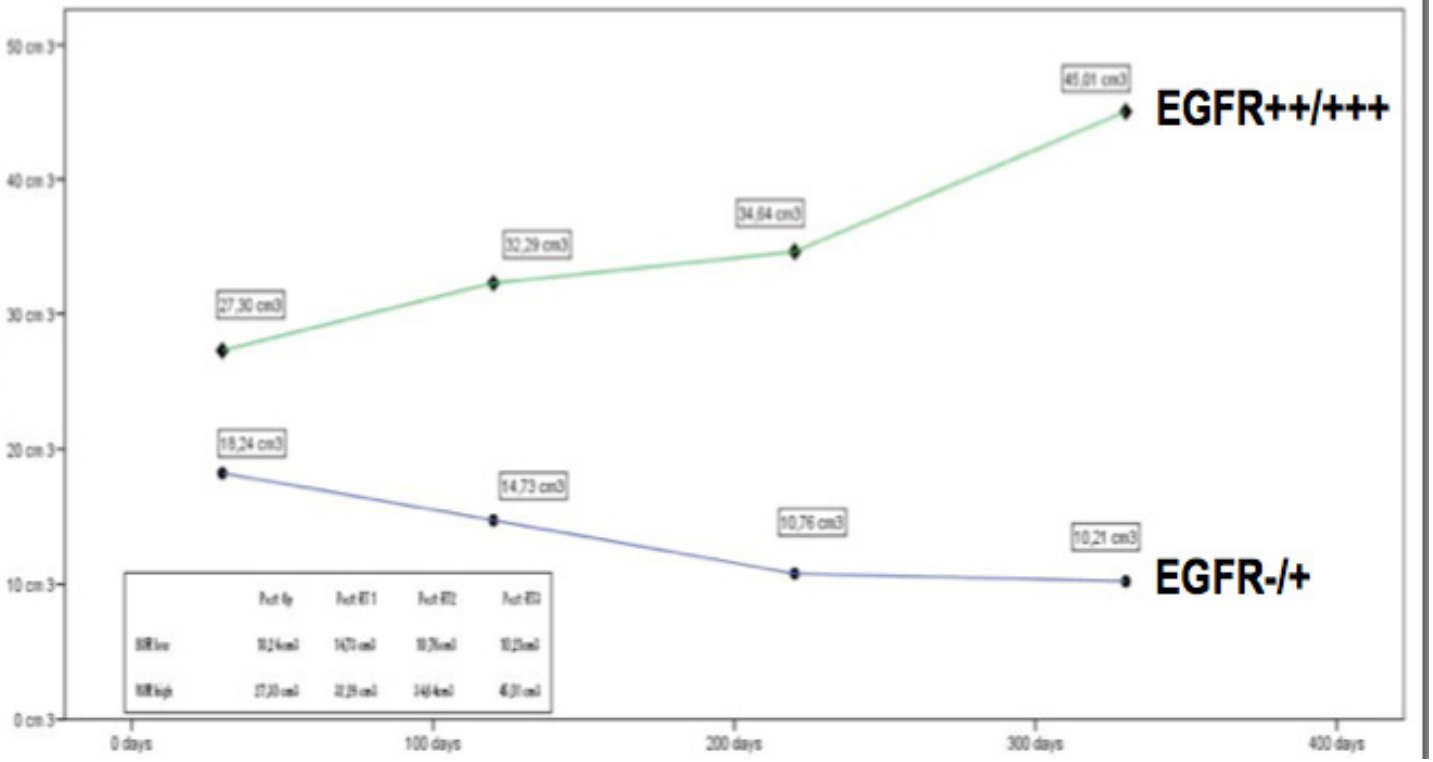
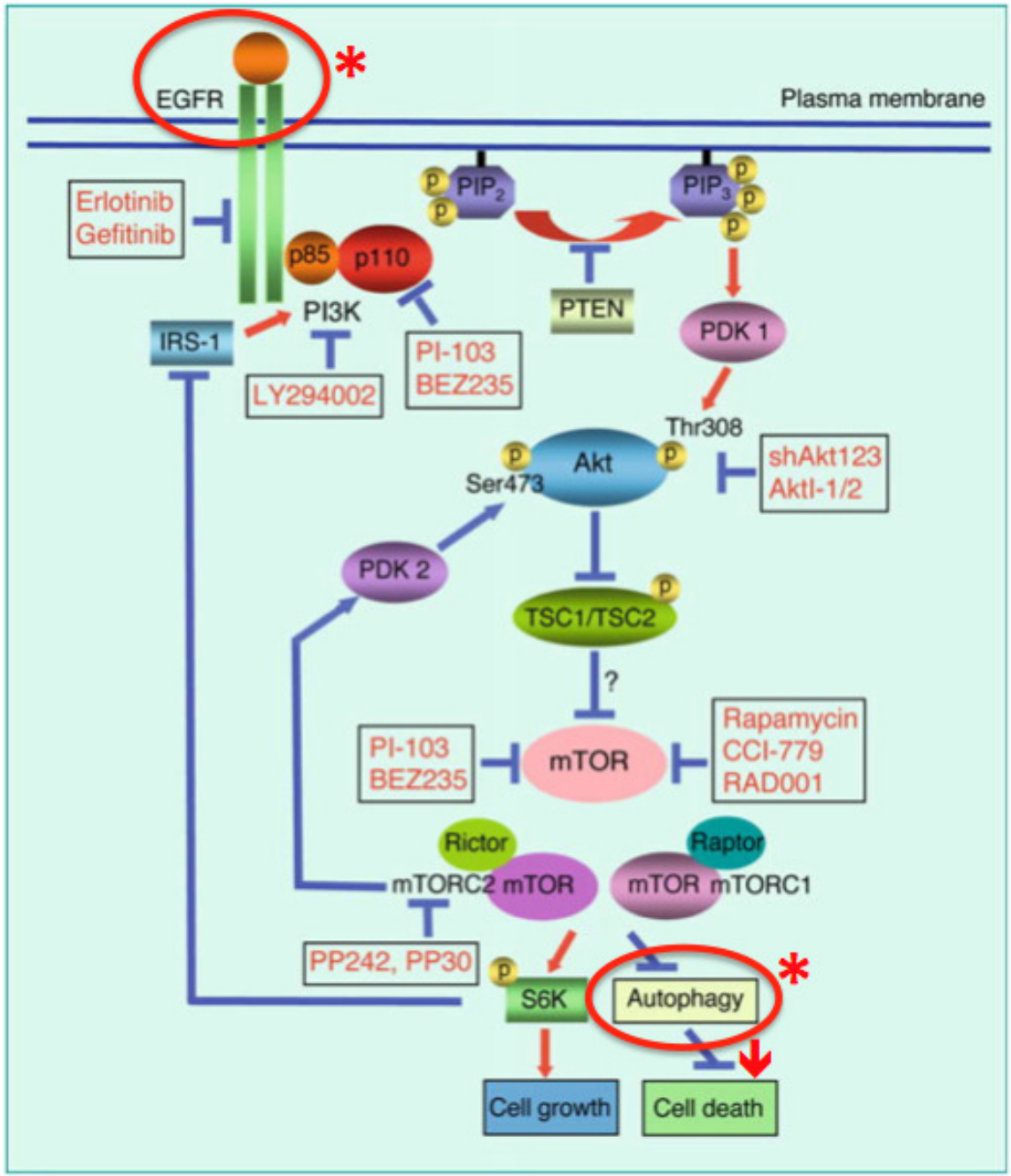


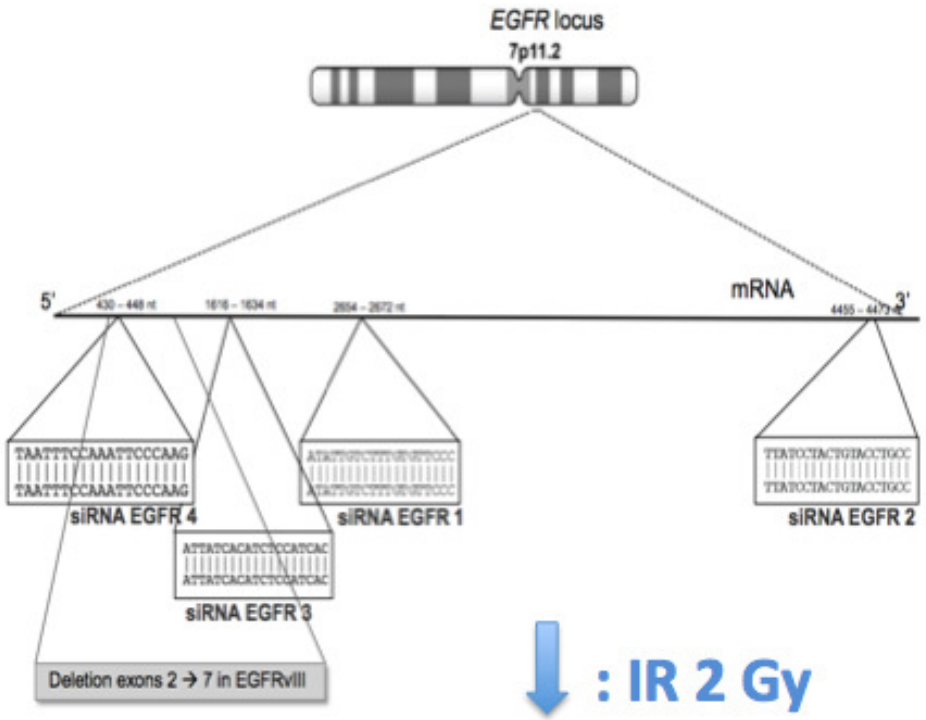


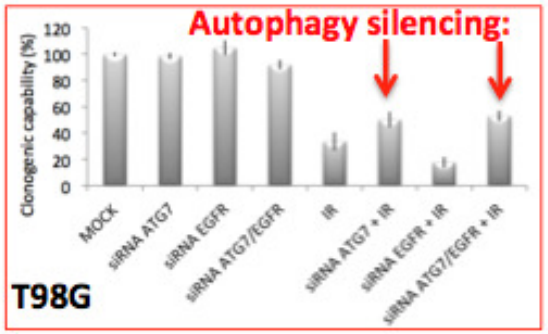
Fan Q-W, Weiss WA:
Targeting the RTK-PI3K-mTOR Axis in Malignant Glioma: Overcoming Resistance
Curr Top Microbiol Immunol. 2010; 347: 279–296.

***:**

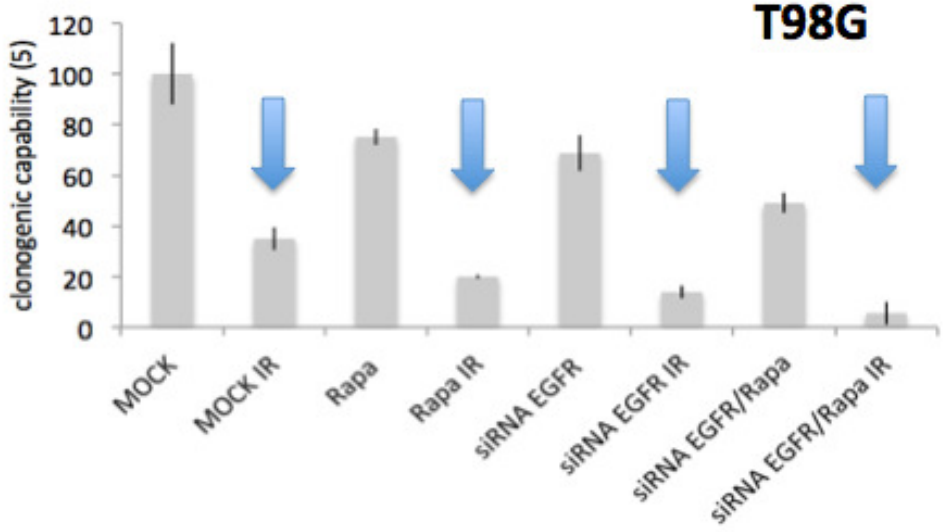
A main role of Autophagy in the EGFR-PI3K-Akt-mTOR axis can also be demonstrated

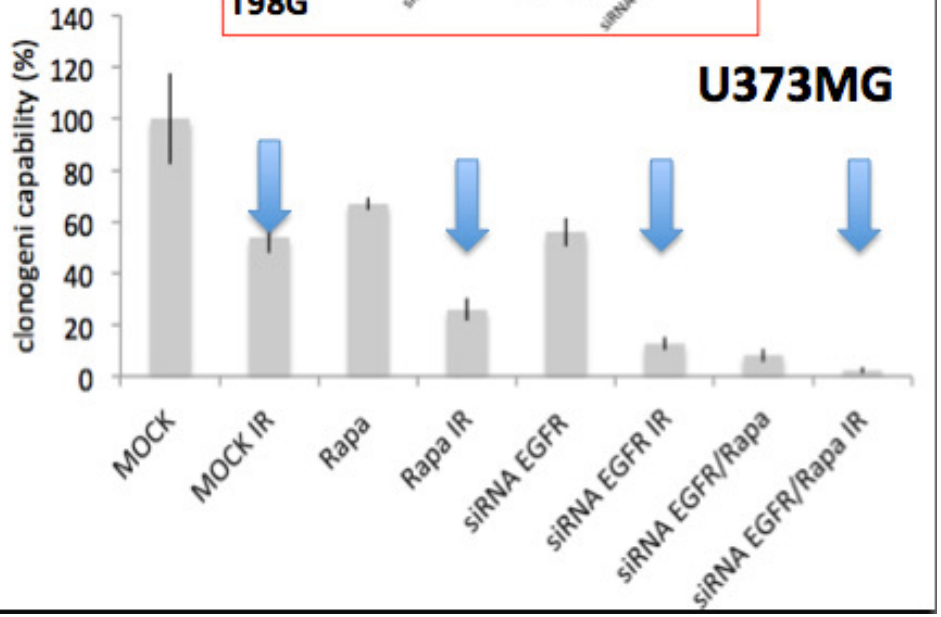
EGFR silencing by a four siRNA pool, mTOR inhibition by Rapamicyn, and IR (2 Gy). Clonogenic tests in T98G and U373MG cell lines.
(S. Palumbo, P. Tini, L. Pirtoli et Al., 2013, unpublished results).





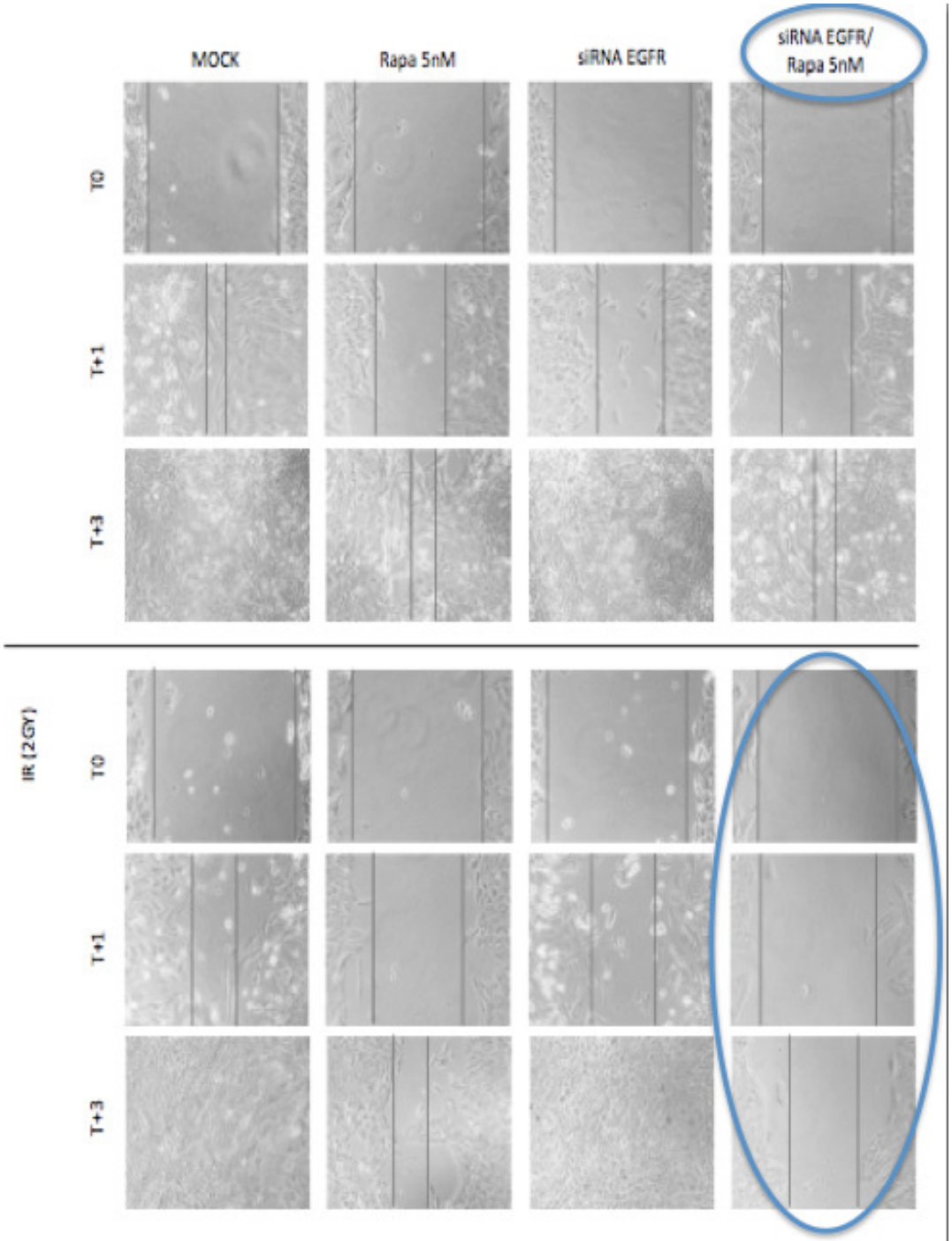
: IR 2 Gy





EGFR **silencing by a four siRNA pool, mTOR inhibition by Rapamicyn, and IR (2 Gy). Cell migration tests in T98G cell line.**
(S. Palumbo, P. Tini, L. Pirtoli et Al., 2013, unpublished results).

IR 2 Gy :



Glioblastoma: From Molecular Pathology to Targeted Treatment

Timothy F. Cloughesy,[1] Webster K. Cavenee,[2,3] and Paul S. Mischel[2,3,4]

[1]Department of Neurology and Neuro-Oncology Program, University of California, Los Angeles, California 90095; email: pmischel@ucsd.edu

[2]Ludwig Institute for Cancer Research, [3]Moores Cancer Center, [4]Department of Pathology, University of California, La Jolla, California 92093

REVIEWS IN ADVANCE

ARI 23 July 2013 16:16

If mTOR Is Such a Compelling Glioblastoma Target, Why Did Rapamycin Fail in the Clinic? Of Feedback Loops and Cross-Talk Pathways

Antitumor Activity of Rapamycin in a Phase I Trial for Patients with Recurrent PTEN-Deficient Glioblastoma

Tim F. Cloughesy[1☯], Koji Yoshimoto[2☯¤], Phioanh Nghiemphu[1☯], Kevin Brown[3], Julie Dang[2], Shaojun Zhu[2], Teli Hsueh[4], Yinan Chen[4], Wei Wang[5], David Youngkin[3], Linda Liau[6], Neil Martin[6], Don Becker[6], Marvin Bergsneider[6], Albert Lai[1], Richard Green[7], Tom Oglesby[5], Michael Koleto[5], Jeff Trent[3], Steve Horvath[8], Paul S. Mischel[2,4☯], Ingo K. Mellinghoff[4☯], Charles L. Sawyers[9☯*]

1 Department of Neurology, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, United States of America, **2** Department of Pathology and Laboratory Medicine, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, United States of America, **3** Translational Genomics Research Institute, Phoenix, Arizona, United States of America, **4** Department of Molecular and Medical Pharmacology, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, United States of America, **5** Taylor Technology, Princeton, New Jersey, United States of America, **6** Department of Neurosurgery, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, United States of America, **7** Department of Neurology, Kaiser Permanente; Los Angeles, California, United States of America, **8** Department of Biostatistics and Human Genetics, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, California, United States of America, **9** Department of Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York, United States of America

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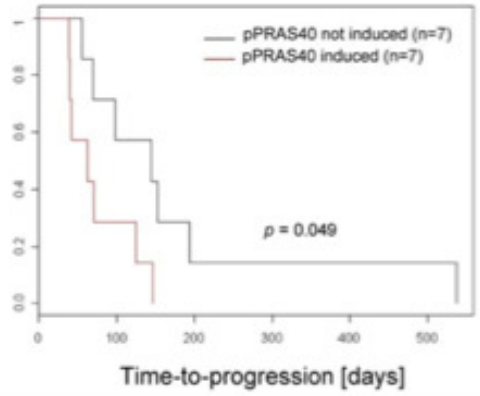
January 2008 | Volume 5 | Issue 1 | e8



The mTOR Signalling Pathway in Human Cancer, Pópulo H et Al.
Int. J. Mol. Sci. **2012,** *13,* 1886-1918

Table 2. mTOR inhibitors in clinical trials.

mTOR inhibitors	Mechanism of action	References
Rapamycin and analogues		
Deforolimus	Binding to the immunophilin FKBP12 Partial mTORC1 inhibitor Cell-type specific mTORC2 inhibitor	[206]
Everolimus	Binding to the immunophilin FKBP12 Partial mTORC1 inhibitor Cell-type specific mTORC2 inhibitor	[206]
Sirolimus	Binding to the immunophilin FKBP12 Partial mTORC1 inhibitor Cell-type specific mTORC2 inhibitor	[206]
Temsirolimus	Binding to the immunophilin FKBP12 Partial mTORC1 inhibitor Cell-type specific mTORC2 inhibitor	[206]
Small molecule inhibitors of kinases		
AZD8055	ATP competitive inhibitor of mTOR	[207]
Ku-0063794	Specific mTORC1 and mTORC2 inhibitor	[208]
PP242	mTOR kinase inhibitor	[201]
PP30	mTOR kinase inhibitor	[201]
Torin1	mTOR kinase inhibitor	[202]
WYE-354	ATP competitive inhibitor of mTOR	[209]
mTOR and PI3K dual-specificity inhibitors		
NVP-BEZ235	ATP-competitive inhibitor of PI3K and mTOR	[205]
PI-103	ATP competitive inhibitor of DNA-PK, PI3K and mTOR	[210]
PKI-179, PKI-587	ATP competitive inhibitor of DNA-PK, PI3K and mTOR	[211,212]
XL765	ATP-competitive inhibitor of DNA-PK, PI3K and mTOR	[203]

Lab Experiences with IR:

Kuger 2013
Pravo 2008

Improving IR- and TMZ- sensitivity by Autophagy manipulation might be achieved also downstream the mTOR pathway, by targeting miRNAs.

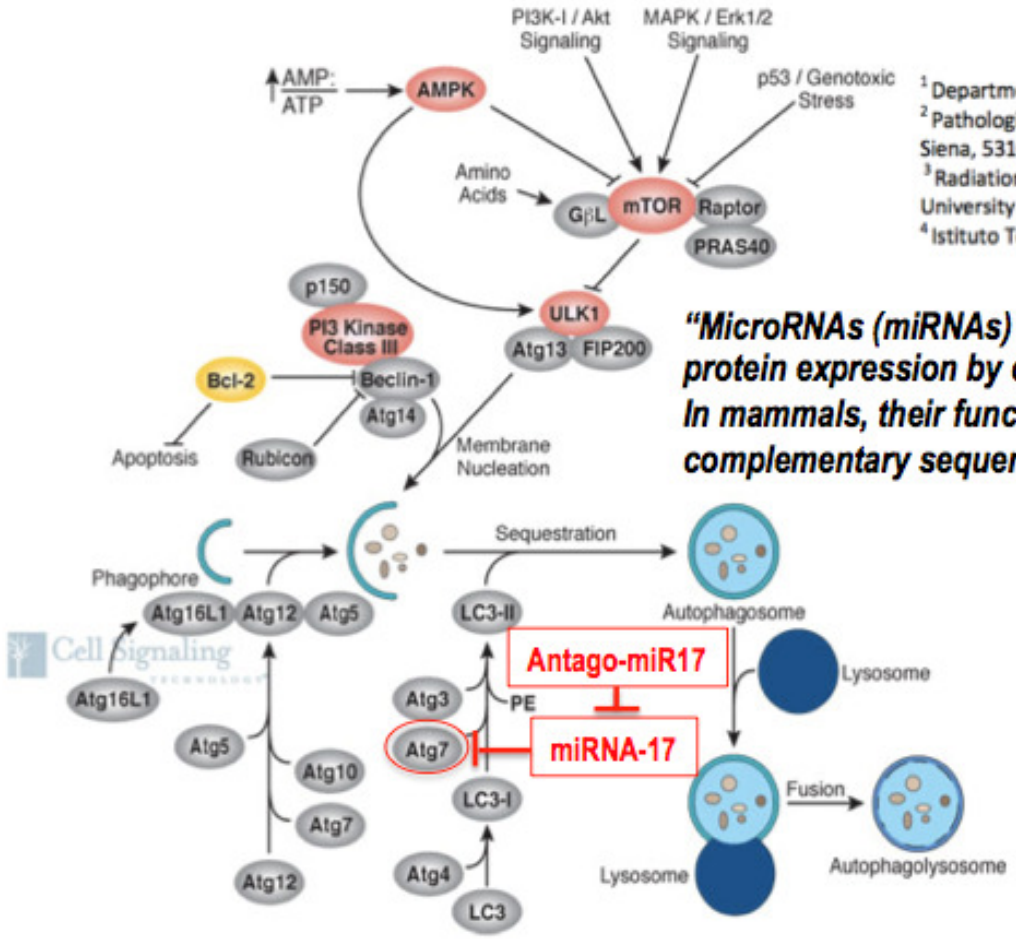
Review Article

J Cell Physiol, 2013, in press.

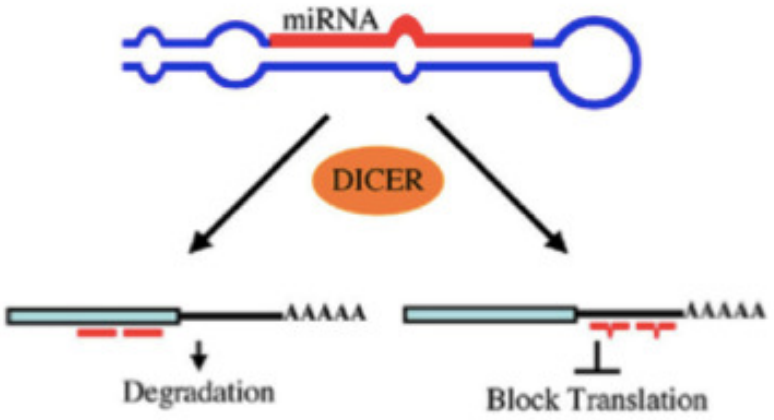
Emerging roles of microRNA in modulating cell-death processes in malignant glioma†

Silvia Palumbo[1*], Clelia Miracco[2,4], Luigi Pirtoli[3,4], Sergio Comincini[1]

[1] Department of Biology and Biotechnology, via Ferrata 1, University of Pavia, Pavia 27100, Italy
[2] Pathological Anatomy Unit, Dept. of Medicine, Surgery and Neuroscience-, University Hospital of Siena, 53100 Siena, Italy
[3] Radiation Oncology Unit, Dept. of Medicine, Surgery and Neuroscience, University Hospital of Siena, 53100 Siena, Italy
[4] Istituto Toscano Tumori, 53100 Siena, Italy



"MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate protein expression by cleaving or repressing the translation of target mRNAs. In mammals, their function mainly represses the mRNA transcripts via imperfect complementary sequences in the 3'UTR of target mRNAs."



RESEARCH PAPER

Cancer Biology & Therapy 14:7, 1–13; July 2013; © 2013 Landes Bioscience

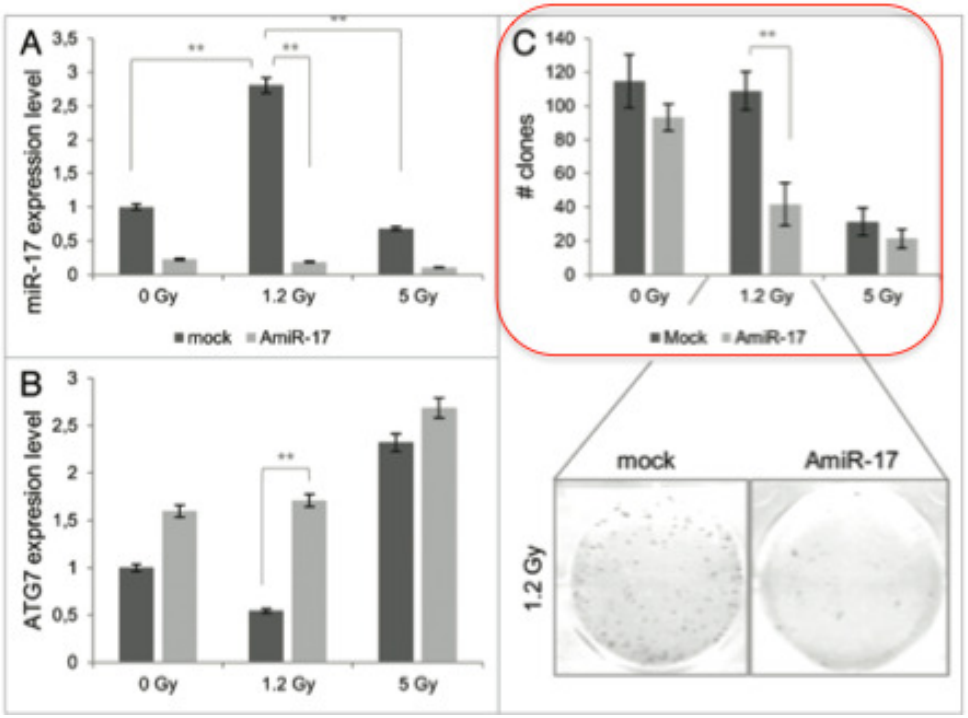
microRNA-17 regulates the expression of ATG7 and modulates the autophagy process, improving the sensitivity to Temozolomide and low-dose ionizing radiation treatments in human glioblastoma cells

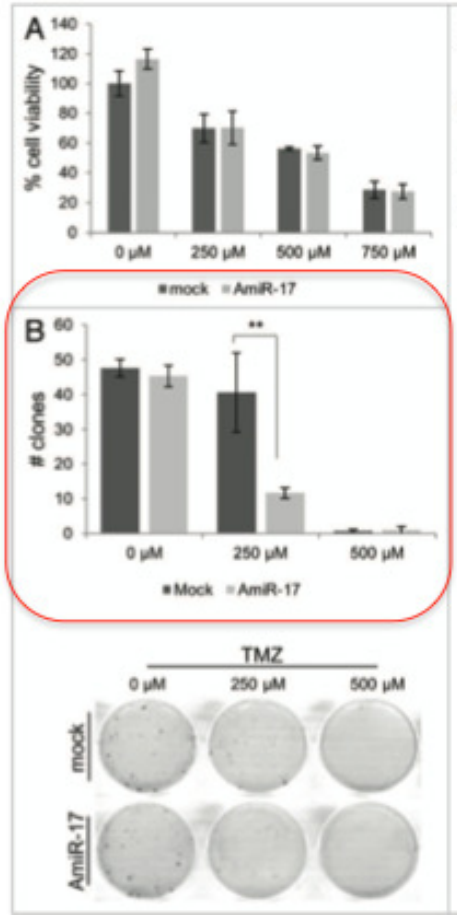
Sergio Comincini,[1,*] Giulia Allavena,[1] Silvia Palumbo,[1] Martina Morini,[1] Francesca Durando,[1] Francesca Angeletti,[1] Luigi Pirtoli[2,3] and Clelia Miracco[2,3]

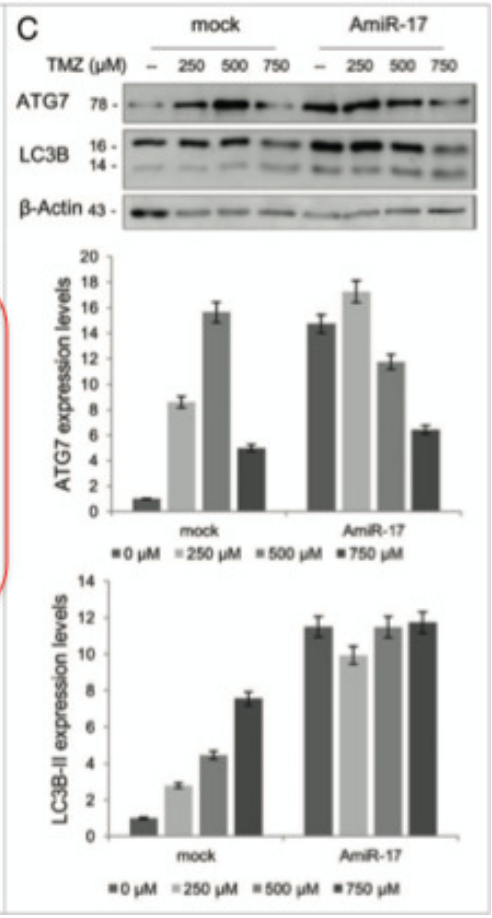
[1]Dipartimento di Biologia e Biotecnologie; Università di Pavia; Pavia, Italy; [2]Dipartimento di Scienze Mediche; Chirurgiche e Neuroscienze; Policlinico Le Scotte; Università di Siena; Siena, Italy; [3]Istituto Toscano Tumori; Firenze, Italy

Antago-miR17 improves ATG7 - LC3B-II expression level and TMZ sensitivity In the TMZ-resistant T98G cell line.

Antago-miR17 improves ATG7 expression level and IR sensitivity mainly at low-dose in the IR-resistant U373MG cell line.







Conclusions:

During the last 3 decades, Molecular - Mechanistic RB has replaced (or integrated) Math Modeling, paralleling the improved knowledge in Pathobiology.

Autophagy (ATG) is a bio-molecular mechanism involving all the domains of Mechanistic RB; it can act both as a pro-survival and a pro-death mechanism. Intra-cellular autophagy signal pathways are actively investigated in GB. Increased suggestion exists of a main cell-death role of ATG in sensitizing GB to IR and TMZ, both on clinical and lab grounds.

Enhanced ATG seems to enhance IR sensitivity mostly at low IR doses and dose-rates, thus suggesting new RT modalities in the clinical setting, in combination with ATG modulating agents.

ATG is involved also in GF signaling (e.g.: EGFR), that can be modulated by ATG manipulation in order to counteract some aggressive behaviors of GB, e.g.: high growth rate and invasiveness.

However, feed-back escape mechanisms have been documented, from the main ATG induction strategy (that is, mTOR inhibition) and unsatisfactory results can be anticipated of clinical trials using Rapalogs associated with IR. This might be obviated by dual PI3K-mTOR inhibitors, and/or by targeting the ATG pathway downstream along the mTOR cascade (e.g.: by inhibiting by antagonists miRNAs acting against ATG genes expression).

The complex pathobiology of GB makes this disease an elusive one in respect of therapy, and a great deal of further study is necessary in molecular and translational RB of GB.