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Overexpression of IKKβ is correlated with cytoplasmic expression of p21CIP1/WAF1 in human breast cancers

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IKKβ was recognized as one of the two catalytic subunits of the IKK complex, which also comprises IKKβ, the other catalytic subunit, and the regulatory subunit IKKβ/NEMO. Constitutive activation of IKK has been reported in various human primary cancers and cancer cell lines. Cellular localization plays a critical role to determine the function of p21, i.e. nuclear p21, as a cell cycle inhibitor, represses cell growth; cytoplasmic p21, as an anti-apoptotic factor, enhances cell survival. Regulation of cytoplasmic p21CIP/WAF1 (p21) is of great clinical significance in molecular oncology due to its identification as an antiapoptotic factor, a poor survival predictor as well as drug-resistance inducers. In an attempt to further characterize the function of IKKβ in cancer development and progression, we investigated the association between IKKβ and other important molecules involved in cell proliferation, cell cycle regulation, and antiapoptotic process by IHC studies of human breast carcinoma specimens. A retrospective study of the immunohistochemical profiles of 128 human primary breast cancers showed that increased total and cytoplasmic p21 expression were highly associated with the expression of IKKβ. The causal relationship study based on cultured cell lines, MDA-MB-453 and MCF-7, confirmed that IKKβ overexpression did upregulate protein levels of total and cytoplasmic p21. Mechanistic investigation demonstrated that IKKβ increased p21 expression through upregulation of p21 mRNA level. Moreover, by western blot, IKKβ was found to be able to upregulate Akt phosphorylation on Ser 473. This novel finding indicated that IKKβ could mediate cytoplasmic p21 accumulation via activation of its downstream target Akt, which was known to phosphorylate p21 and lead to cytoplasmic localization of p21.

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RAD001 (everolimus) in vivo sensitivity is associated with wild-type PTEN function in a panel of serially transplanted GBM xenografts

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Background: mTOR inhibitors (RAD001 – everolimus – and CCI-779) have shown promising activity in glioblastoma multiforme (GBM). However, the molecular features that predict for in vivo sensitivity to mTOR inhibition have not been fully characterized. Therefore, we evaluated the RAD001 sensitivity of a panel of human GBM xenografts that were initially established and subsequently maintained by serial passage in nude mice.
Materials and Methods: U87 and U251 GBM cell lines and 17 primary GBM xenograft lines were tested in an orthotopic therapy model. Mice with established intracranial tumors were randomized to therapy with 0 or 10 mg/kg RAD001 delivered 5 days/week by oral gavage for at least 4 weeks. Three lines also were tested with RAD001 in combination with 20 Gy radiation therapy (RT; 2 Gy bid × 10 fractions). Efficacy of therapy was determined through a log-rank analysis of the survival data.
Results: The survival analysis in relation to key molecular features is shown in the table below. RAD001 treatment prolonged survival in 4 of the primary GBM xenograft lines (GBM10, 15, 22, and 28) and in U251 tumors. Interestingly, 4 out of 5 of the sensitive tumor lines have wild-type PTEN and a corresponding hypo-phosphorylation of Akt. In the sensitive GBM10 line with wild-type PTEN, RAD001 treatment was associated with a 61% reduction in MIB1 labeling ($p < 0.001$), while in the resistant GBM14 line with mutant PTEN, RAD001 treatment resulted in an insignificant reduction of MIB1 labeling of 17% ($p = 0.17$). In 2 of the sensitive GBM10 and GBM22 lines, RAD001 combined with RT significantly prolonged survival as compared to either treatment alone: median survival following treatment with placebo, RT, RAD001, RT+RAD001 for GBM10 was 48, 51, 55 and 72 days, respectively ($p = 0.0007$) and for GBM22 was 44, 77, 53, 127 days, respectively ($p = 0.02$).
Conclusions: RAD001 extends survival in a quarter of xenograft lines tested, and combinations of RAD001 with radiation may further enhance the efficacy of therapy. However, contrary to in vitro studies, our results suggest that wild-type PTEN function is associated with mTOR inhibitor sensitivity.

GBM	Survival prolongation (%)	log rank test	EGFR status	PTEN
6	11	0.94	vIII	wt
8	4	0.75	WT	HD
10	15	0.01	no	wt
12	6	0.83	WT	wt
14	0	0.48	no	mutant
15	14	0.03	WT	wt
16	27	0.96	no	HD
22	23	0.03	no	wt
26	−13	0.59	WT	HD
28	12	0.04	no	mutant
34	9	0.79	WT	wt
36	−1	0.42	no	HD
38	2	0.29	WT	wt
39	16	0.23	vIII	wt
43	0	0.82	no	wt
44	36	0.84	no	wt
46	11	0.10	no	NA
U87	6	0.75	no	mutant
U251	63	0.007	no	wt

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Erlotinib sensitivity in GBM xenografts is associated with EGFR amplification and PTEN status

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Background: Selective small molecule EGFR inhibitors have demonstrated clinical efficacy in some glioblastoma tumors with EGFR amplification. Unfortunately, studying the mechanisms of sensitivity in this subset of GBM tumors has been difficult due to a lack of established GBM cell lines with amplified EGFR. To address this problem, we have established a panel of human GBM xenografts that are maintained by serial passage in the flanks of nude mice, and in this model, patient tumor EGFR amplification status is maintained.
Methods: This model system has been used in the current study to identify erlotinib-responsive GBMs, and to investigate potential mechanisms of sensitivity to erlotinib. For each of 13 xenograft lines examined, mice with established intracranial tumors were treated with daily erlotinib (M-F) for a minimum of 4 weeks.

GBM#	Survival prolongation (%)	p-value	EGFR amplification	PTEN	p53
6	25	0.53	vIII	wt	mutant
8	4	0.80	WT	deleted	wt
10	10	0.30	no	wt	wt
12	47	0.0007	WT	wt	mutant
14	13	0.73	no	mutant	wt
15	8	0.07	WT	wt	wt
16	54	0.45	no	deleted	wt
22	26	0.39	no	wt	mutant
28	6	0.69	no	mutant	mutant
34	0	0.77	WT	wt	wt
36	−10	0.37	no	deleted	mutant
39	33	0.006	vIII	wt	wt
44	−2	0.36	no	wt	wt

Results: Survival was significantly prolonged in 2 of the 13 xenograft lines (GBMs 12 and 39), and both of these lines had wild-type PTEN expression and amplified EGFR. To further evaluate the mechanisms of erlotinib effect in EGFR amplified tumor lines, mice with established intracranial or flank tumor of GBM lines 6, 12, 15 and 39 were treated for 5 days with erlotinib or placebo and then flank tumors or whole brains were resected for analysis. For intracranial xenografts, erlotinib treatment reduced MIB-1 proliferation values by 20%, 19%, 21% and 78%, respectively ($p < 0.02$ for each line).