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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).

In contrast, drug treatment had no significant effect on CD-31 microvessel density or on the induction of apoptosis as assessed by TUNEL staining. Western blot analysis of flank tumor specimens revealed that erlotinib treatment consistently suppressed phosphorylation of S473-Akt in all lines, while there were minimal effects on phospho-Erk.
Conclusions: These data are consistent with the idea that inhibition of downstream signaling through the PI3K/Akt signaling pathway in PTEN wild-type tumors leads to suppression of tumor proliferation and are consistent with the importance of specific GBM genotypes for the anti-proliferative effects of erlotinib in human GBMs.

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Combined targeting of mTOR and ILK for maximal suppression of cancer cell growth, Akt activation and cell survival

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Constitutive activation of the PI3Kinase signaling pathway is a frequent event in many types of cancers and results in increased cell growth and survival. There is currently intense interest in this pathway, and Pi3Kinases, mTOR and Akt are the major therapeutic targets. mTOR exists in two different complexes: TORC1, which is rapamycin sensitive, regulates protein translation and cell growth, and TORC2 which is rapamycin-insensitive and apparently regulates Akt phosphorylation and activation. Although rapamycin and its analogs are in clinical trials, a major conundrum of TORC1 inhibition is that Akt phosphorylation and cell survival are stimulated due to the inhibition of the normal feedback inhibition of PI3Kinase activation by TORC1. Effective targeting of this pathway therefore requires inhibition of both TORC1 (to block cell growth), and also Akt activation to block cell survival.
Using a combination of siRNA-mediated knock-down and pharmacological inhibitors, we have found that knock-down of Integrin-Linked Kinase (ILK) (an upstream regulator of Akt Serine-473 phosphorylation), or Rictor, a component of TORC2, suppress Akt phosphorylation and induce apoptosis. However, complete functional knock-down of mTOR in breast or prostate cancer cell lines results in a stimulation of Akt phosphorylation. Similarly, treatment with rapamycin also results in increased Akt phosphorylation. Knock-down of either Rictor or ILK, or both, completely suppresses this increased Akt phosphorylation resulting from mTOR inhibition, suggesting that ILK and Rictor regulate Akt phosphorylation in an mTOR-independent manner. Interestingly, ILK and Rictor can be co-immunoprecipitated suggesting a potential interaction between these two proteins. Importantly, treatment of breast and prostate cancer cells with a combination of rapamycin and a highly selective small molecule ILK inhibitor, QLT-0267, results in the complete suppression of Akt and p70S6Kinase, inhibition of cell growth and survival in vitro, and significant tumor growth delay in in vivo xenograft models.
Our results suggest that combined mTOR and ILK targeting effectively shuts down activated PI3Kinase pathway and suppresses tumor growth.

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Insulin-like growth factor-I secreted from prostate stromal cells mediates tumor-stromal cell interactions of prostate cancer

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Solid tumors are composed of tumor cells and surrounding stroma including extracellular matrix, fibroblasts, macrophages, and endothelial cells. The growth of tumor cells is regulated by the stromal cells through diffusible factors and their adhesion. These tumor-stromal cell interactions significantly contribute to the growth and metastasis of some cancers such as the breast and prostate cancers. Prostate cancer shows high expression of type I insulin-like growth factor receptor (IGF-IR) and prostate stromal cells produce insulin-like growth factor-I (IGF-I). Although high plasma level of IGF-I is related to the development of prostate cancer, the significance of prostate stromal IGF-I in the regulation of the prostate cancer remains elusive. Here we show that the stromal IGF-I certainly regulates the development of prostate cancer. Coinoculation of prostate stromal cells (PrSC) increased the growth of human prostate cancer LNCaP and DU-145 tumors in SCID mice. The conditioned medium of PrSC, as well as IGF-I, induced phosphorylation of IGF-IR and increased the growth of LNCaP and DU-145 cells. PrSC, but not LNCaP and DU-145 cells, secreted significant amounts of IGF-I. Coculture with PrSC increased the growth of DU-145 cells in vitro, but the pretreatment of PrSC with siRNA of IGF-I did not enhance it. Furthermore, various chemical inhibitors consisting of 79 compounds with about 60 different targets led to the finding that only IGF-IR inhibitor suppressed the PrSC-induced growth enhancement of DU-145 cells. Thus, these results show that prostate stromal IGF-I mediates tumor-stromal cell interactions of the prostate cancer to accelerate tumor growth, supporting the idea that the IGF-I signaling is a valuable target for the treatment of the prostate cancer.

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Complexity of constitutive NF-kappaB activity as a therapeutic target in breast cancer cells

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Constitutive activation of NF-κB is common in breast cancer and provides a potential therapeutic target. We compared the mechanisms of activation in un-stimulated breast cancer cell lines. The effect of specific cell signalling inhibitors on NF-κB activity (24 hrs treatment), mRNA levels of NF-κB-regulated genes for IκBα and metastatic biomarkers Bcl-xL, ICAM-1, IL-6 and uPA (30 hrs), viability and apoptosis (48 hrs) were compared in HBL100, MDA-MB-468 and MDA-MB-231 lines. NF-κB activity was measured by reporter gene expression. mRNA levels of downstream targets were examined by real-time PCR. NF-κB subunits were detected by immunoblotting. Nuclear extracts of HBL100 and MDA-MB-231 cells contained NF-κB p65, p50, p105, p52, p100, RelB and RelC proteins, whereas expression of p105 and RelC differed in the extracts of MDA-MB-468 cells. The level of constitutive NF-κB activation was in the order HBL100 $\ll$ MDA-MB-468 < MDA-MB-231, with no correlation to IκBα mRNA levels or nuclear levels of NF-κB family members. Aggressively metastatic MDA-MB-231 cells also showed the highest levels of mRNA expression of the biomarkers Bcl-xL, IL-6 and uPA, consistent with their metastatic potential. Expression of uPA mRNA in all three cell lines correlated with invasiveness. Upstream inhibition using specific inhibitors for PI3K, Src, EGFR, IKKβ or proteasomal degradation resulted in cell line-specific effects on NF-κB-regulated reporter gene activity. Inhibition of PI3K with LY294002 or proteasomal degradation with MG132 had the most prominent effect on NF-κB activity in all cell lines. The altered patterns of reporter gene expression did not correlate with viability in response to any treatment. Nor was there any correlation between expression of the reporter gene and endogenous NF-κB target genes. MG132 increased cell death in all three lines. It increased IL-6 mRNA levels and decreased mRNA levels of uPA and anti-apoptotic Bcl-xL in MDA-MB-231 and HBL100 cells, however NF-κB reporter gene expression was increased in HBL100 cells and decreased in MDA-MB-231 cells. NF-κB activity was inversely correlated with IκBα expression in LY294002-treated HBL100 cells, but showed no correlation in the other cell lines. In MDA-MB-468 cells, PI3K inhibition resulted in increased NF-κB reporter gene activity and decreased IL-6 mRNA, whereas proteasomal inhibition decreased NF-κB reporter gene activity and increased mRNA levels of metastatic biomarkers ICAM-1 and uPA. These data show that the NF-κB pathway is regulated in a complex way, probably, by several members of the NF-κB family, which results in cell-specific modulation of expression of NF-κB–regulated reporter gene and endogenous downstream target genes. Modulation of this pathway has a significant effect on expression of several metastatic biomarkers.

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MDM2 309T>G polymorphism in human sarcomas

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Background: MDM2 protein regulates p53 activity and stability through the inhibition of p53-dependent transcription and its proteosomal degradation. Further, MDM2 regulates the location of p53 by transporting p53 out of the nucleus into the cytoplasm. The presence of the SNP 309T>G in the promoter region of the MDM2 gene, determines an increased level of the MDM2 protein with an attenuation of the p53 tumour suppressor activity. This genetic variant may have an important role in the pathogenesis of sarcomas, either for soft tissue sarcomas (STS) or osteosarcomas. The aim of this study is to investigate the role of the polymorphism 309T>G of the MDM2 gene in the risk of developing sarcomas.
Methods: Three hundred fifty-two patients affected by sarcomas (150 patients with STS and 202 patients with osteosarcomas) have been tested on genomic DNA from peripheral blood mononuclear cells or frozen tumour tissue. The genotype analysis was performed using the Pyrosequencing a mini-sequencing technique. The relative risk (OR, 95% CI) associated with the polymorphism MDM2 309T>G in developing sarcomas was investigated by matching the frequencies among patients with those of a healthy population of 487 individuals.
Results: Among all the cases enrolled in our study, the frequencies were 34.1% for the homozygous wild type TT (120 pts), 44.0% for the heterozygous TG (155 pts) and 21.9% for the homozygous polymorphic variant GG (77 pts). A significant difference was observed in frequencies