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Inhibition of the mammalian target of rapamycin sensitizes U87 xenografts to fractionated radiation

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Rapamycin is a highly selective inhibitor of mTOR signaling pathways, and rapamycin analogs are currently being evaluated in Phase I and II clinical trials as single agent therapy for recurrent GBM. The mammalian target of rapamycin (mTOR) modulates key signal transduction pathways in malignant gliomas that promote cell survival, tumor proliferation and angiogenesis, and these cellular processes may contribute to the clinical radioresistance of glioblastomas. Therefore, in anticipation of possibly using rapamycin in primary GBM patients, the combination of rapamycin and radiation was evaluated in U87 malignant glioma xenografts in a regrowth delay assay. Compared to sham irradiation, treatment of U87 flank xenografts with 4 Gy $\times$ 4 fractions delivered over 18 days was ineffective in these radioresistant tumors (regrowth delay of -0.2 $\pm$ 3 d, mean $\pm$ 95% CI). In contrast, radiation combined with rapamycin was significantly more effective than rapamycin alone (regrowth delay 19.1 $\pm$ 6.3 d). Rapamycin also sensitized U87 xenografts to a single 11 Gy dose of radiation, although the degree of sensitization was less profound than with fractionated radiation. This rapamycin-mediated sensitization could result from disruption from either tumor- or host-dependent processes. To distinguish between these possibilities, the combination of rapamycin and radiation was evaluated *in vitro* in U87 spheroids and monolayer culture. Similar to the xenograft studies, fractionated radiation alone had minimal effect on spheroid regrowth compared to control treatment (regrowth delay of 5.0 $\pm$ 3.8 d), while radiation combined with rapamycin was significantly more effective than rapamycin alone (regrowth delay of 10.2 $\pm$ 3.2 d). Interestingly, rapamycin had no effect on clonogenic survival following a single dose of radiation in U87 cells growing in monolayer culture.

Taken together, these data suggest that rapamycin disrupts an mTOR-dependent signaling pathway important for recovery following ionizing radiation specifically in cells growing in the context of a solid tumor mass either *in vivo* or *in vitro*. More importantly, these results provide a strong rationale for the clinical evaluation of the combination of rapamycin and radiation in patients with glioblastoma multiforme.

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Transcriptional response to ionizing radiation (IR) in normal and tumor cells

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The objective of radiation therapy is to deliver a lethal dose to cancer cells but attenuate the toxic effects of IR on adjacent normal tissue. Undesirable sequels of radiotherapy are the development of tumor resistance and normal tissue damage. Profiling of RNA on DNA arrays provide a method to study the response of thousands of genes to ionizing radiation. We used this approach to profile the transcriptional response to various doses of irradiation in primary human embryonic lung (HEL) fibroblasts and the U87 human malignant glioblastoma cell line and in U87 tumor xenograft implanted in mice. Doses ranged from 1 to 3 Gy, which are commonly employed in radiotherapy to 10 Gy which is frequently employed to study biochemical responses of mammalian cells to IR and in radiosurgery of some brain tumors. Data acquisition and filtration were optimized with receiver operator characteristic (ROC) approach for control and reduction of false positive signals. Data confirmation was based on semi-quantitative conventional RT-PCR and real-time PCR using SYBR Green. Data showed relatively low complexity of transcriptional response to ionizing radiation in the terms of temporal patterns of genes, induced by different doses. More then 90% of all responding genes were separated in 4 major expressional clusters. Functional classification showed non-random distribution of different functional groups in different expressional clusters. This analysis may give temporal specificity to intervention strategies by identifying genes or clusters with specific temporal patterns that may be selected as molecular targets for radiotherapy modification. An example of such a gene is Epidermal Growth Factor Receptor (EGFR). We observed a 4-5 fold induction of the EGFR gene following exposure to 1 or 3 Gy, at the 1 or 5 hour time points, and 6-7 fold induction at all doses at 24 hours. Anti-EGFR agents such as tyrosine kinase inhibitors currently employed in the clinical setting, can be combined with a defined ionizing radiation regimen to enhance its effect. Data suggest that modification of radiotherapy regimens to account for both radiation-dose and timing specificity of cellular responses may significantly enhance clinical outcome of IR treatment. Data also provide valuable information for the selection of possible targets for optimized temporally -modulated combined radiotherapy.

Apoptosis

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Phosphorylation on Tyrosine-15 of p34Cdc2 by ErbB2 Receptor Tyrosine Kinase Inhibits p34Cdc2 Activation and Is Involved in Resistance to Taxol-Induced Apoptosis

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Overexpression of p185ErbB2 confers resistance to taxol-induced apoptosis by inhibiting p34Cdc2 activation but the inhibitory mechanisms are not fully understood. Here we report that the inhibitory phosphorylation on tyrosine (Tyr)15 of Cdc2 (Cdc2-Tyr15-p) is elevated in ErbB2-overexpressing breast cancer cells and primary tumors independent of Wee1, Cdc25C, and p21Cip1. ErbB2 can bind to and co-localize with cyclin B-Cdc2 complexes and phosphorylate Cdc2-Tyr15. The ErbB2 kinase domain is sufficient for binding to and directly phosphorylating Cdc2 specifically on Tyr15. Increased Cdc2-Tyr15-p in ErbB2-overexpressing cells corresponds with delayed M-phase entry. An ErbB2 tyrosine kinase inhibitor and a kinase-dead ErbB2 inhibited Cdc2-Tyr15-p. ErbB2-overexpressing cells expressing the non-phosphorylatable Cdc2Y15F mutant are more sensitive to taxol-induced apoptosis than cells not expressing Cdc2Y15F. Thus, ErbB2 membrane RTK may directly phosphorylates Cdc2, which operates in the nucleus.

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Expression of nuclear BCL10 is highly correlated with the expression of nuclear NF-kB and is predictive of helicobacter pylori-dependent status in early-stage high-grade gastric malt lymphoma

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Background: We have recently demonstrated that around 60% of early-stage high-grade gastric mucosa-associated lymphoid tissue (MALT) lymphomas are rendered durable tumor remission by eradication of H pylori (J Clin Oncol 2001; 19:4245 -51). However, the histologic and molecular features that help predict the H pylori-dependent state of these tumors remains elusive. The intracellular signal protein BCL10 was identified by its direct involvement in t(1;14)(p22;q32) of MALT lymphomas, and is a putative regulator of antigen-receptor-mediated NF-kB activation. Upregulation of BCL10 may trigger a constitutive NF-kB signal from the antigen receptor, and therefore may contribute to antigen-independent growth and progression of gastric MALT lymphoma.

Purpose: The present study sought to investigate the correlation between nuclear BCL10 expression and nuclear NF-kB expression, and the correlation of their expression with the tumor resistance to H pylori eradication therapy in patients with stage IE high-grade gastric MALT lymphoma.

Materials and Methods: Lymphoma biopsies of all patients, who had participated in a prospective study of H pylori-eradication for stage IE high-grade gastric MALT lymphoma, were collected. The H pylori-dependent status was verified by the results of the prospective clinical trials. There were 13 patients with H pylori-dependent, and 7 patients with H pylori-independent high-grade gastric MALT lymphoma. The expression of BCL10 and NF-kB in pre-treatment paraffin-embedded lymphoma tissues was determined by immunohistochemistry with anti- BCL10 antibody (polyclonal; 1:10; Santa Cruz Biotechnology) and anti-NF-kB RelA (p65; 1:150; Santa Cruz Biotechnology). A semi-quantitative method was used to determine the level of expression of RelA. Reactive spleen and lymph nodes tissue sections were used as the control. The co-expression of nuclear BCL10 and NF-kB activity was further analyzed by a confocal immunofluorescence microscopy.