the present study was to evaluate boronated EGF and the anti-EGFR MoAb cetuximab (IMC-C225), as molecular targeting agents for BNCT of the F98 rat glioma. The parental wildtype tumor, $F98_{WT}$, was transfected with the gene encoding human EGFR to produce a receptor (+) glioma, designated $F98_{EGFR}$. Boronated bioconjugates were produced by linking a heavily boronated polyamidoamino (PAMAM) dendrimer by means of heterobifunctional reagents to either EGF or cetuximab. Biodistribution studies were carried out in Fischer rats bearing intracerebral (i.c.) implants of $F98_{EGFR}$ or $F98_{WT}$ gliomas and the bioconjugates were administered by either direct intratumoral (i.t.) injection or convection enhanced delivery (CED). At 24 h following i.t. injection of boronated cetuximab (C225-G5-B_{1100}), the mean tumor boron concentrations in rats bearing either $F98_{EGFR}$ or $F98_{WT}$ gliomas were 92.3±23.3 μg/g and 36.5±18.8 μg/g, respectively. In contrast, uptake of the non-targeted boronated dendrimer (G5-B_{1100}) was 6.7±3.6 μg/g. Based on its favorable *in vivo* uptake, C225-G5-B_{1100} was evaluated as a delivery agent for BNCT in $F98_{EGFR}$ glioma bearing rats. This was carried out at the Massachusetts Institute of Technology Nuclear Reactor (MITR) 24 h following CED of C225-G5-B_{1100} and 2.5 h after i.v. boronophenylalanine (BPA). The mean survival time (MST) of rats that received the bioconjugate, administered i.c. by CED, was 45±3 d compared to 25±3 d for untreated control animals. A further enhancement in MST to >60 d was obtained by administering C225-G5-B_{1100} and 2.5 h after i.v. BPA ($p<0.001$). Similar studies were performed using boronated EGF (BD-EGF), administered by CED to $F98_{EGFR}$ glioma bearing rats. The MST of rats that received BD-EGF either alone or in combination with i.v. BPA were 53±13d and >61±14 d, respectively, compared to 40±5 d for BPA alone and 31±4 d for irradiated controls ($p<0.001$). These data are the *first* to demonstrate the efficacy of a boronated MoAb for BNCT of an i.c. glioma and are paradigmatic for future studies using combinations of low and high molecular weight ^{10}B delivery agents.

643 POSTER

Radiosensitization of human prostate cancer by natural polyphenol inhibitor of Bcl-2/XL, (−)-gossypol, results in tumor regression

L. Xu[1], D. Yang[1], S. Wang[1], W. Tang[1], M. Liu[1], J. Chen[1], T. Lawrence[2], M.E. Lippman[1]. [1]*University of Michigan, Internal Medicine, Ann Arbor, MI, USA;* [2]*University of Michigan, Radiation Oncology, Ann Arbor, MI, USA*

Radioresistance markedly impairs the efficacy of tumor radiotherapy and involves anti-apoptotic signal transduction pathways that prevent radiation-induced cell death. The Majority of human prostate cancers overexpress Bcl-2 and/or Bcl-XL, the important negative regulators of apoptosis. Overexperssion of Bcl-2 and/Bcl-xL in prostate and other types of cancer cells has been shown to confer resistance to radiation and chemotherapeutic agents. (−)-gossypol, a natural product from cottonseed, has recently been identified as a potent small molecule inhibitor of both Bcl-2 and Bcl-XL. In the current study, we tested our hypothesis that (−)-gossypol may improve prostate cancer's response to radiation by inhibiting the anti-apoptosis activity of Bcl-2/XL and making cancer cells more sensitive to radiation therapy.

Our data show that (−)-gossypol inhibits tumor cell growth and induces apoptosis in human prostate cancer PC-3 cells with a high levels of Bcl-2/XL proteins, but has minimal effect on normal cells. In clonogenic assays, treatment of PC-3 cells with (−)-gossypol significantly reduced radiation resistance of PC-3, resulted in 10- and 20-fold reduction of colony formation at 8 Gy X-ray irradiation. Fluorescence resonance energy transfer (FRET) assay using Bcl-XL-CFP and Bax-YFP or Bad-YFP co-transfected DU-145 cells suggests that (−)-gossypol potently blocks the interaction of Bcl-xl with Bax and Bad in live cells, in a time- and dose-dependent manner. The data support that (−)-gossypol induces apoptosis, at least in part, through inhibition of the anti-apoptotic protein Bcl-XL, although the interactions of (−)-Gossypol with other targets, either directly or indirectly, may also play a role and this is a subject of our further investigations.

Our in vivo studies using PC-3 xenograft models in nude mice show that orally administered (−)-gossypol has anti-tumor activity but achieves a much greater efficacy with tumor regression when used in combination with X irradiation. Combination therapy of (−)-gossypol 10 mg/kg, p.o. q.d.5 × 4 weeks, with fractionated irradiation, 2 Gy q.d.5 × 3 weeks, achieved 96% tumor growth inhibition (T/C = 3.4%) in tumors with initial size of 100 mm^3, significantly more effective than either (−)-gossypol or radiation alone (T/C = 96% and 37%, respectively) ($p<0.01$, n = 16). Similar results were observed with PC-3 tumors with initial sizes of 200 and 400 mm^3 at the start of radiation, whereas only combination therapy resulted in tumor regression. For PC-3 tumors with starting size of 200mm3, (−)-gossypol plus radiation achieved significant tumor growth delay (T-C = 54.5 days) as compared with (−)-gossypol or radiation alone (T-C = 0 and 8.5 days, respectively). In situ TUNEL-staining showed significantly more apoptotic cells induced in the tumors treated with (−)-gossypol plus radiation than either treatment alone. Anti-CD31 immunohistochemical staining indicates that (−)-gossypol plus radiation significantly inhibited the tumor angiogenesis.

In summary, our results demonstrate that the natural polyphenol inhibitor of Bcl-2/xl, (−)-Gossypol, can radiosensitize prostate cancer *in vitro* and *in vivo* without augmenting toxicity. (−)-Gossypol may improve the outcome of current prostate cancer radiotherapy and represents a promising novel anticancer regime for molecular targeted therapy of hormone-refractory prostate cancer with Bcl-2/XL overexpression. Clinical trials are being planned.

644 POSTER

Selective inhibition of Ras, PI3-kinase and Akt isoforms can radiosensitize human carcinoma cell lines

E.J. Bernhard[1], I. Kim[1], K.A. Cengel[1], S.S. Bae[2], A. Fernandes[1], J. Wu[1], R.J. Muschel[3], W.G. McKenna[1], M.J. Birnbaum[2]. [1]*University of Pennsylvania, Radiation Oncology, Philadelphia, USA;* [2]*University of Pennsylvania, Howard Hughes Medical Institute, Philadelphia, USA;* [3]*Children's Hospital of Philadelphia, Pathology, Philadelphia, USA*

H-Ras activation has been shown to enhance survival after irradiation in many tumor cells. Human tumors more frequently have mutations in K-*ras* or demonstrate Ras activation due to signaling from receptor tyrosine kinases such as the EGF receptor (EGF^R). The role of K-Ras in radiation resistance and the relative contributions of H- and K- and N-Ras signaling to EGF^R–mediated promotion of cell survival are not as well defined. We have now examined the effects of selectively inhibiting the expression of individual Ras isoforms on downstream signaling and radiation survival using RNA interference (RNAi) in a panel of human tumor cell lines that differ in Ras status. We also used this technique to examine the contribution of PI3-kinase and Akt isoforms to radiation survival. Specific inhibition of oncogenic K-Ras expression in cells expressing $K\text{-}Ras^{V12}$ and specific inhibition of oncogenic H-Ras expression cells expressing $H\text{-}Ras^{V12}$ decreased clonogenic survival after irradiation. Inhibition of H- or N-Ras, but not K-Ras reduced clonogenic survival in cells with EGF^R-activated Ras signaling. Inhibition of H-, K-, or N-Ras using siRNA decreased both phospho-Akt and phospho-p42/44 MAP kinase, however, pharmacologic inhibition of the MEK-ERK pathway by itself had little effect on survival while inhibition of PI3-kinase resulted in radiosensitization. Isoform-specific inhibition of PI3-kinases was carried out with siRNA. Combined inhibition of PI3-kinase p85β and either p110α or p110β subunits had a greater effect on radiation survival than inhibition of individual subunits. Testing the contribution of Akt isoforms showed that Akt-1 was the most effective in down-regulating phospho-Akt and decreasing radiation survival. However, co-transfection of siRNA for Akt-1, -2 and -3 further decreased radiation survival. The effects of siRNA inhibition of Akt-1 were rescued by exogenous expression of mouse Akt-1. This study demonstrates that the activation of PI3K-Akt pathway is an important part of survival signaling by Ras, whether the activation results from mutation of *ras* or over-expression of EGF^R. This study further indicates that specific inhibition of PI3-kinase and Akt isoforms can reverse survival signaling in tumor cells.

645 POSTER

Radiation sensitization of lung cancer through inhibition of MDM2

B. Lu, C. Cao, K. Niermann, D. Hallahan. *Vanderbilt University, Radiation Oncology, Nashville, USA*

Background: MDM2 interacts with p53 and reduces its transcriptional activity and stability. Inhibition of MDM2 using antisense oligonucleotides results in radiosensitization by up-regulating p53, p21 and bax or down-regulating bcl-2. The purpose of this study was to define additional mechanisms of radiosensitization via inhibition of MDM2 and determine whether inhibition of MDM2 enhances the cytotoxic effects of radiation upon lung cancer and its tumor vasculature.

Methods: Antisense oligonucleotides against MDM2 were used to downregulate MDM2 expression. H460 lung cancer cells were transiently transfected with either a control oligonucleotides or the antisense oligonucleotides, using Lipofectin. Western Blotting was used to verify specific attenuation of the target gene expression in the transfected cells. They were then treated with or without 3Gy of radiation. At various time points following irradiation, H460 cells were assayed for their survival by clonogenic assay; for apoptosis by flow cytometry of stained apoptotic cells; and for cell senescence by quantification of beta-galactosidase-expressed cells. Biological effects of inhibiting MDM2 were determined using H460 xenografts. H460 cells were injected into the hind limb of nude mouse to establish the mouse model for lung cancer. Mice bearing tumors were treated with seven i.p. injections of antisense oligonucleotides at the daily dose of 10mg/kg. They were treated with nothing else or with 2Gy × 5