Intrinsic and irradiation-induced tumor selectivity of liposome-based gene therapy targeting Akt activation.

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The vascular network is a highly accessible target for tumor therapy. However, as for any cancer treatment, the primary goal is to deliver sufficient amounts of drug to the targeted tissue while minimizing damage to healthy organs. Cationic liposomes have been identified as delivery systems significantly more effective at targeting tumor *versus* normal vascular networks. Though, the liposome uptake by the liver restricts their potential as shuttles to selectively target the tumor endothelium. Here, we reasoned that additional selectivity could be found in the nature of the delivered gene and by combining another anti-tumor therapy. Accordingly, the pro-survival PI3K /Akt pathway is thought to be activated by ionizing radiations and a dominant-negative Akt would therefore mostly target tumors (versus non-irradiated organs). Furthermore, we have recently documented that irradiation led to NO-mediated tumor vessel dilation which could thereby enhance the access of liposomes to the tumor. In this study, we have therefore examined whether radiotherapy and the use of dominant-negative Akt plasmid delivered by cationic liposomes could mutually improve their efficacies. We first used cultured endothelial cells and isolated tumor microvessels to demonstrate that low dose irradiation led to the stimulation of both Ser^{473} Akt and Ser^{1177} eNOS phosphorylations (e.g., activation); the use of a PI3K inhibitor further indicated that the former largely accounted for the increased NO/cGMP production. Using a reporter-encoding plasmid, we then showed that irradiation dramatically enhanced the *in vivo* expression of the tagged protein in the tumor endothelium. Also, using $eNOS^{-/-}$ mice, we documented the key role of NO in mediating the adjuvant effects of X-Ray on plasmid delivery, likely through an increase in tumor blood flow. We then combined local irradiation to the liposome-dominant negative Akt DNA complex administration. In two different protocols associating gene therapy with either a single 6 Gy dose or a 5 x 2 Gy fractionated scheme, we consistently observed synergistic effects of the combinatory treatment. In fact, the transgene when administered alone, did not reveal any tumor response and the tumor growth delay after irradiation represented less than 50% of the gain obtained when combining both approaches ($n=8$; $P<0.01$); these findings were obtained in two mouse tumor models. In conclusion, the combination of low dose radiotherapy and liposome-cargoed dominant-negative Akt gene therapy appears particularly well suited to selectively target tumor vasculature. Besides the intrinsic tumor specificity of local X-Ray administration and the propensity of cationic liposomes to bind tumor vessels, we have further identified a "treatment-driven" selectivity, e.g. the ability of radiotherapy to induce Akt activation in tumor vasculature and to increase the liposome access to the tumor.