Results: In prostate (PC-3) tumors treated with 224Ra wires (20-40 KBq), average tumor volume of the tumors was three times smaller than that of the inert wire group, including one case of complete cure with no tumor recurrence. A similar effect was observed for 224Ra wire-treated human GBM-derived (U87-MG) tumors (3-4 mm in diameter), where the average tumor volume of the 224Ra group, after 25 days of treatment, was 10 times smaller compared to inert group. Insertion of a single 224Ra wire into lung SCC-derived (NCI H520) tumors (125 mm³ average volume) reduced their volume by 41% compared to the inert wire-treated tumors at 20 days post treatment. Larger (606 mm3 volume) human-derived SCC tumors (CAL 27) treated by several 224Ra wires (150-230 kBq per tumor) shrunk by 80% from their original size within 14 days of treatment, while the inert wire-treated tumors continued to increase in size. The exposure of all tumor cells to alpha particles reduced their viability in tissue culture in a dose-dependent manner.

Conclusions: In vivo studies showed that DART can effectively destroy human-derived tumors, and in vitro tests indicated that tumor cell death is a result of a direct effect of alpha particles. Tumor destruction may be augmented by damage to intratumoral blood vessels. DART could potentially be combined with chemotherapy or other treatment modalities to effectively treat non-resectable tumors.

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Sensitization of cancer cells to radiation using hybrid nanoparticles - activation of apoptotic factors

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Introduction: Nanotechnology-based tools and techniques are rapidly emerging in the field of molecular imaging diagnostics and targeted drug/gene delivery, a field that is expected to generate many innovations and play a crucial future role in medicine. In the past 3 years, several leading groups in “nanomedicine” have reported a great potential of hybrid nanoparticles, consisting of semiconductors quantum dots (QDs) and conventional photosensitizers (e.g., phthalocyanines, eosins, natural pigments, etc.) or QDs and X-ray photosensitizers, in radiation-induced photodynamic therapy of cancer. The nanoparticles can also have a promising application be conjugated with cancer-selective ligands that should minimize side-effects on normal cells and tissues.

In this study, we describe the selective sensitization of cancer cells to laser irradiation, using several QD-based hybrid nanoparticles, conjugated with cluster-of-differentiation antibodies specific for leukemia cells.

Methods: The experiments were performed on cultured cells, derived from patients with acute lymphoblastic leukemia or chronic myeloid leukemia, as well as on normal lymphocytes, derived from healthy volunteers. The cells were incubated with QD probes over different time-intervals (up to 48 hours). Interaction of the QDs with the cells was detected by fluorescent confocal microscopy. After incubation, the cells were subjected to laser irradiation (red light). The cell viability was analyzed before and after irradiation, using flow cytometry. The apoptosis was analyzed using fluorescein-labeled Annexin-V, activation of caspase cascade, and DNA fragmentation tests. The generation of reactive oxygen species was detected using EPR spectroscopy.

Results: Fluorescent confocal imaging demonstrated that QD-based hybrid nanoparticles interact specifically with leukemia cells, but do not interact with normal lymphocytes. Without laser irradiation, the nanoparticles did not affect significantly cell viability. However, the viability of QD-treated leukemia cells markedly decreased (~25-40%, depending on the chemical structure of the QD probe) after laser irradiation, while the viability of normal lymphocytes remained at the control level. Acceleration of free radical generation and induction of apoptosis (with activation of caspase enzymes and DNA-fragmentation) were also detected in the cancer cells. In normal lymphocytes, we observed only a slight reversible expression of phosphatidylserine on the cell surface (detected by Annexin-V) after QD treatment and subsequent laser irradiation.

Conclusions: The results suggest that QD-based hybrid nanoparticles manifest target-selective laser-induced cytotoxicity due to specific interaction with leukemia cells. The mechanism of QD induced cancer cell death is due to acceleration of free radical generation and induction of apoptosis.

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Implication of HIF-1 and NF-kB in the radiosensitizing effect of gefitinib on human malignant glioma xenografts

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Background: The radiosensitizing effect of anti-EGFR therapies has been reported on preclinical glioma models and several clinical trials are currently ongoing. However, molecular mechanisms leading to this radiosensitization have not been clearly described.

EGFR activation triggers multiple signal transduction pathways, leading to the activation of two transcription factors: the nuclear factor êB (NF-êB) and the hypoxia inducible factor-1 (HIF-1), implicated in tumour angiogenesis, tumour growth and radioresistance.

The aim of the present work was to determine whether HIF-1 and NF-êB are involved in the radiosensitizing effect of gefitinib (Iressa®), an EGFR tyrosine kinase inhibitor.

Methods: Fragments of the malignant glioma TCG3 were heterotopically implanted into nude mice. When tumour volume reached 250±50 mm3, mice were randomly assigned in four groups: CTRL, radiotherapy (RT), gefitinib (GEF) and GEF+RT. Radiotherapy was delivered as daily fractions of 2 Gy. Gefitinib was injected intraperitonealy at 75 mg/kg/day. Treatments were administered for 5 consecutive days and tumours were excised 48H after the end of treatments. Antitumoural activity of treatments was investigated. Expression of activated HIF-1 and NF-êB were measured by an ELISA assay and target genes expression (VEGF and TNFα) were determined by qRT-PCR. VEGF protein levels were measured by an ELISA assay.

Results: Our results showed that the concomitant association GEF+RT produced a significant greater antitumour effect than RT or GEF alone: mean tumour volumes were 39mm3; 933mm3 and 76mm3 respectively.

In CTRL group, both transcription factors HIF-1 and NF-êB were constitutively activated as compared to human healthy brain.

Whatever the treatment, expression of activated HIF-1 was not modified while qRT-PCR experiment showed a significant decrease in VEGF mRNA level for the RT+GEF tumour group, as compared to other groups (p =.0003). This data was confirmed by protein expression analysis.

Considering the NF-êB pathway, we showed that activated p50 level was significantly enhanced by fractioned radiotherapy (p =.014) and that gefitinib was unable to reduce radiation-induced p50 expression. However, the radiation-induced TNFα mRNA level was considerably decreased when RT was combined with GEF (p =.0002).

Conclusions: This study demonstrates that radiosensitizing effect of GEF is associated with a major decrease of VEGF and TNFα, but mechanisms leading to this decrease need further investigations.

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HIF-1a modulates hypoxic radioresistance in vitro

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Hypoxia-inducible factor-1 (HIF-1) is a transcription factor that upregulates target genes under hypoxic conditions. HIF-1 overexpression in tumour biopsies is correlated with treatment failure and mortality. Here we examined the role of HIF-1a in oxygen-dependent radiosensitivity. Subconfluent HeLa cells were subjected to hypoxia (<0.1% O2) for 1 h before irradiation at 6.2Gy. Cell proliferation measured 7 days later using resazurin reduction and cyQUANT® assays was compared to that of cells irradiated under normoxia. HIF-1a in whole cell lysates was measured by ELISA. HIF-1a knockdown using RNA interference was measured 72 h after transfection. Hypoxia induced a two-fold increase in the levels of HIF-1a detected, with irradiation under hypoxia causing an almost 3-fold increase in the levels of HIF-1a compared with control normoxic levels. HIF-1a prolyl hydroxylation inhibitors (dimethyloxaloylglycine 500μM and ethyl-3,4 dihydroxybenzoate 500μM) increased HIF-1a levels in both normoxic and hypoxic cells, as well as reducing the effect of irradiation on subsequent cell proliferation. Conversely, diacylglycerol kinase inhibitor R59949 (200μM) and HSP90 inhibitor 17-allylamino-demethoxygeldamycin (6μM) both reduced HIF-1a levels in normoxic and hypoxic cells. They also increased the radiosensitivity of hypoxic cells. Echinomycin (5nM), an inhibitor of HIF-1a/DNA binding, enhanced irradiation-induced injury in hypoxic cells. In normoxic cells, echinomycin reduced the effect of irradiation on cell proliferation. RNAi reduced HIF-1a levels to below 20% of controls and significantly enhanced the radiosensitivity of hypoxic cells.Collectively, HIF-1a levels were inversely related to radiosensitivity under hypoxia and normoxia, but the effects were more prominent in hypoxic cells, possibly because HIF-1a levels were higher. These data suggest that HIF-1a is a major determinent of radiosensitivity in HeLa cells. Hence pharmacological manipulation of HIF-1a signalling may provide a tool to improve the efficacy and selectivity of tumour radiation therapy.We gratefully acknowledge Aid Cancer Treatment, Cork, for financial support and Cancer Research Ireland for an Oncology Scholars Travel Award to EMcL.