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Cyclo-oxygenase inhibitors enhance hypoxic radiosensitivity in some human solid tumour cell lines through COX-2-dependent mechanisms

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Radiosensitization by COX-2 selective inhibitors has been reported mainly at suprapharmacological concentrations, so COX-2 independent mechanisms cannot be ruled out. The involvement of COX-2 in hypoxic radioresistance also remains unclear. Here we tested the role of COX-2 in hypoxic radioresistance in vitro using pharmacological concentrations of COX inhibitors in parallel with RNA interference. Treated and untreated cervical cancer HeLa, breast cancer MCF-7 and melanoma MeWo cell lines were irradiated at 6.2 Gy under normoxic and hypoxic conditions (<0.1% O2 x 1h) then subcultured and allowed to proliferate. Seven days later, reduction of resazurin to resorufin was used as an index of cell growth.

COX-1 inhibitor SC560 (5μM) did not affect radiosensitivity under either normoxia or hypoxia in any of the cell lines tested. In contrast, COX-2 selective inhibitors NS398 (10μM) and SC791 (1μM) sensitized HeLa and MCF-7 cells irradiated under hypoxia but not normoxia. MeWo cells were unaffected. This radiosensitization did not correlate with COX-2 levels in the cell lines as measured immunochemically (MeWo>>HeLa>MCF7). In HeLa cells radiosensitivity was not affected either by prostaglandin E1 analogue misoprostol (5μM) or phospholipase A2 inhibitor methyl arachidonyl fluorophosphate (50 μM). RNA interference leading to 85% reduction in COX-2 expression did not affect radiosensitivity but attenuated NS398 enhancement of hypoxic radiosensitivity. This inconsistency between pharmacological and genetic targeting of COX-2 most likely reflects different effects on cell-cycle progression.

Hence COX-2 inhibitors at pharmacological concentration selectively increase radiosensitivity under hypoxia, in some but not all solid tumour cell lines. The effect is mediated through COX-2 but other mechanisms may also contribute. Therefore selective inhibition of COX-2 using these agents may provide a useful adjunct to radiation therapy.

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Combined treatment with membrane targeted apoptosis modulators and ionising radiation increases apoptotic signaling at the mitochondria

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Background: Radiotherapy is an integral part of current treatment concepts in the therapy of glioblastoma. The membrane active apoptosis modulators Erucylphosphocholine (ErPC) and Erucylphosphohomocholine (ErPC3) enhance the sensitivity of tumor cells towards radiation-induced apoptosis and improve eradication of clonogenic tumor cells after ionizing radiation (IR). Aim of the present study was to analyse the importance of the intrinsic cell death pathway for the induction of apoptosis after combined treatment (IR + ErPC/ErPC3) and to define the molecular details of combined action at the mitochondria.

Methods: The importance of the intrinsic pathway for the induction of apoptosis after combined treatment settings was analysed in a human glioblastoma cell line (T98G) as well as in a Jurkat T-cell lymphoma model (Bax negative, p53-negative). Functional relevance of Bcl-2-proteins for efficacy of combined action was checked in Jurkat cells with (Jurkat Bak-positive) versus without (Jurkat Bak-negative) expression of Bak as well was with (Jurkat Bcl-2) versus without (Jurkat vector) over-expression of Bcl-2. Apoptotic levels and cell cycle distribution were measured by FACS-analysis (mitochondrial membrane potential + DNA-content). Cleavage of caspases and expression patterns of different Bcl-2 family proteins after combined treatment were analysed by Western Blotting.

Results: The results obtained in the Jurkat T-cells revealed a dependence of poapoptotic action of combined treatment on the pro-apoptotic Bcl-2 protein Bak: While over-expression of Bcl-2 only delayed and decreased induction of apoptosis after combined treatment with ErPC3 and ionizing radiation, Bak-deficiency completely blocked activation of caspases and execution of apoptosis. In T98G cells the proapoptotic BH3-only proteins Bim, Noxa and Puma were up regulated by combined treatment, whereas proapoptotic Bax and Bad were activated. Similar changes were also observed in Jurkat cells. However, in Jurkat cells down-regulation of antiapoptotic Mcl-1 was a central event for cell death induction.

Conclusion: Combined efficacy of radiotherapy and ErPC/ErPC3 involves specific changes in the balance of pro- and antiapoptotic proteins at the mitochondrial level fostering apoptosis induction through the intrinsic pathway.

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Intratumoral 224Ra-loaded wires combined with chemotherapy can destroy solid malignant tumors of various histological types in mice and prolong survival

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Objectives: Alpha particle radiation is highly lethal for cancer cells but has so far not been used in the treatment of solid tumors due to its short range in tissue. We have developed a new approach in which tumors are treated with intratumoral 224Ra-loaded wires that continually release by recoil short-lived alpha-emitting atoms. These disperse in the tumor and deliver a lethal dose over a region measuring 3-7 millimeters in size. The proposed method was termed Diffusing Alpha-emitters Radiation Therapy (DART). The present study examines the curative effects of a combination between the 224Ra-loaded wires and anti-tumor chemotherapy, against tumors of various histological types.

Methods: Tumor cells from pancreatic (Panc02), squamous cell (SCC) (SQ2), and colon carcinomas (CT26), were injected subcutaneously into mice. Stainless steel 224Ra-loaded wire(s) (0.3 mm-diameter and 3-5 mm long) were inserted under anesthesia into tumors of 4-10 mm in diameter. Cisplatin, Gemcitabine, or 5-FU were administered concurrently. Animals were monitored for tumor development and survival. Also, an in-vitro set-up was used to assess killing of cancer cells by alpha particles.

Results: Treatment of squamous cell carcinoma with a regimen of two (5 mg/kg) i.v doses of cisplatin given concomitantly with two 224Ra-loaded wires (11.5-28.1 kBq), caused substantial growth arrest of 93%, and extended survival from 44 to 87 days. The combined treatment reduced both local tumor growth and metastatic spread to the lungs.

Pancreatic tumors in C57BL/6 mice were treated with one 224Ra-loaded wire, in combination with Gemcitabine. The combination of 224Ra wires (13-45 kBq) and Gemcitabine achieved the best local control of tumor growth. Significant reduction in tumor volume was achieved in such treated mice compared to all other treatment groups.

224Ra-loaded wires (15 kBq/wire), inhibited tumor growth of colon adenocarcinoma tumors (4-6 mm) by 45%, and even led to complete cure. Injection of 5-FU (75 mg/kg) with the 224Ra wire (17 kBq) augmented tumor destruction and growth retardation (35% compared to 224Ra and 5-FU alone, and 74% compared to no treatment).

In vitro experiments with all tumor cells exposed to 224Ra atoms revealed a dose dependent killing of the tumor cells. The combined treatment with alpha particles and chemotherapy increased apoptosis and arrested cell proliferation to larger extent than any of the components alone.

Conclusions: Diffusing Alpha-Emitting Radiation Therapy is an effective treatment to treat solid malignant tumors, and can be further potentiated in combination with chemotherapy. This combined treatment modality holds significant potential for the treatment of non-resectable human cancers.

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Radiotherapy of solid malignant human tumors in athymic mice by intratumoral 224Ra-loaded wires releasing alpha emitting atoms can achieve local tumor control

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Objectives: Alpha radiation (high LET of 100-200 keV/micron) is the most lethal form of radiation. Yet, its short range in tissue (<100 microns) has limited its use in the treatment of cancer to radiolabeled pharmaceuticals or antibodies. We developed a new method of intratumoral radiotherapy (brachytherapy) termed Diffusing Alpha-emitters Radiation Therapy (DART), based on insertion of 224Ra-loaded wires, which release by recoil short-lived alpha-emitting atoms. These atoms disperse in the tumor, leading to the formation of a high dose region through their alpha decays, and equivalent areas of tumor destruction. In the present study we examined the ability of 224Ra-loaded wires to destroy and control the development of several human-derived tumors implanted in athymic mice.

Methods: The experiments were performed on athymic mice bearing malignant human- derived tumors including prostate (PC-3), glioblastoma (GBM, U87-MG), lung SCC (NCI H520), breast (MDA-MB-231), and HNSCC (CAL-27). One or more 224Ra-loaded wires (0.3 mm-diameter and 3-5 mm long), were inserted into the tumors, and the mice were followed for tumor growth rate and survival. Tumors treated with inert wires served as controls. In vitro killing of tumor cells by alpha radiation was also assessed, on cells seeded in 96 wells plates implanted with 224Ra or exposed to alpha particle flux from a 228Th source.

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.