Radiobiology

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Human cervix carcinoma: a comparison between hypoxia measured by pimonidazole and invasive pO2 probes. An international multi-center study

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Purpose: To compare hypoxia measured by invasive oxygen electrodes and the hypoxia-specific marker pimonidazole in human cervix carcinoma prior to evaluation of pimonidazole as a prognostic marker of treatment outcome.

Methods: Pre-treatment hypoxia was measured by both assays in 86 patients with primary cervix carcinomas (FIGO stage Ib, n=8; IIa, n=2; IIb, n=39; IIIa; n=3, IIIb; n=23; IVa, n=10 and IVb, n=1). Pimonidazole was given as a single injection (0.5 g/m2 i.v.) and 10-24 hours later tumor pO2 was done using an Eppendorf pO2 Histograph and biopsies were taken, formalin-fixed, paraffin-embedded. Hypoxia was detected by immunohistochemestry using monoclonal antibodies directed against reductively activated pimonidazole. Data were analyzed by a semi-quantitative scoring system and evaluated as the fractions of fields at highest score (pimo 30). Invasive tumor pO2 was evaluated as the fraction of pO2 values less than 10 mmHg (HF10). Necrosis was scored by one observer in HE stained sections and categorized into 4 groups.

Results: Both pimonidazole binding and invasive electrode measurements varied significantly within and between tumors. HF10 ranged from 0-100% (median 72%) and pimo 30 ranged from 0-75% (median 6%). Also, the degree of necrosis was heterogeneous. There was a trend that the most hypoxic tumors measured by oxygen electrodes had the highest score of microregional necrosis, and no pimonidazole binding.

Conclusion: However, there was no statistically significant correlation between pimonidazole and oxygen electrode measurements of hypoxia in these uterine cervix carcinoma(Spearman's rank correlation analysis).

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Patterns of expression of three hypoxia regulated proteins (Hypoxia Inducible Factors HIF1a/HIF1b and Carbonic Anhydrase (CA9) in squamous cell lung carcinoma

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Introduction: Two proteins, the Hypoxia Inducible factors HIF1alpha and HIF2alpha, have been recently identified as key molecules regulating the transcription of a variety of genes related to erythropoiesis, glycolysis and angiogenesis, following hypoxic stimulation. Carbonic anhydrase-9 (CA9), an enzyme involved in the reversible metabolisme of the carbon dioxide to carbonic acid, has been also shown to be regulated by hypoxia through the HIF pathway.

Materials and Methods: Using immunohistochemistry, we evaluated the expression of HIF1a, HIF2a and CA9 proteins in normal lung tissues and in 74 tissue samples from squamous cell lung carcinoma (SqCLC). The ESEE122, the EP190b and the M75 MoAbs were used to detect these proteins, respectively. The degree of necrosis was also assessed as extensive, focal and absent.

Results: HIF1a and HIF2a proteins showed a mixed cytoplasmic/nuclear pattern of expression in cancer cells, tumoral vessels and tumor infiltrating macrophages, as well as in areas of metaplasia, while normal lung components showed negative or very weak cytoplasmic staining. Strong HIF1a and HIF2a expression was noted in 46/74 (62%) and in 33/74 (45%) of cases, respectively. A significant co-expression of these proteins was noted (p=0.002) but, there was no association of HIF with necrosis. CA9 expression was membrane (with or without cyroplasmic expression) and was noted in cancer cells around small or large areas of necrosis. This contrasts the diffuse, necrosis independent, patterns of HIF expression. A significant direct association of CA9 expression with the extent of necrosis was observed (p=0.0008). CA9 expression was mainly identified in tumors overexpressing HIF1a/2a (23/50 vs. 6/24; p=0.12) but the difference was not significant as half of cases with HIF overexpression failed to show CA9 up-regulation. CA9 was not expressed in normal lung tissues.

Conclusions: We conclude that up-regulation of the hypoxia regulated proteins HIF1a, HIF2a and CA9 is a common event in SqCLC. The different patterns of expression suggest that the frames of hypoxia necessary for the induction of these proteins are not identical. Profound hypoxia in the range of tissue necrosis is necessary for the CA9 up-regulation, while lower hypoxia levels seem enough to induce HIF expression. The clinical relevance of these hypoxia related markers in response to radiotherapy, chemotherapy and in the prognosis of cancer patients is under investigation.

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Enhancing radiation therapy when the CO2 content of carbogen is reduced from 5% to 2%

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Purpose: Some patients involved in clinical trials of carbogen (95% O2 + 5% CO2), for reducing tumour hypoxia, experience difficulties breathing the gas. To overcome this it has been suggested that the CO2 content be reduced to 2%. This study was designed to see whether such a reduction was still effective at enhancing radiation response.

Methods: A C3H mammary carcinoma grown in the right rear foot of CDF1 mice was used and treatments performed when at 200 mm3 in size. Restrained, but non-anaesthetised, mice were gassed with either gas mixture at a flow rate of 2.5 l/min for various times before local tumour irradiation (PIBT; pre-irradiation breathing time), with the gassing maintained during the radiation period. Tumour response was assessed using the endpoint of tumour growth time (TGT; time to reach 3 times the treatment volume) and the results presented as means (±1 S.E.) for 8-12 animals.

Results: The TGT for control tumours was 3.8 days (±0.5). This was unchanged by breathing carbogen (with 5% or 2% CO2) alone. For radiation (15 Gy) alone the TGT was increased to 14.4 days (±0.7). With normal carbogen (5% CO2 content) this radiation response was further increased to a maximal TGT of 27.6 days (±5.1) with a PIBT of 5 min., but at longer time intervals the enhancement decreased such that with a PIBT of 60 min. the TGT was 21.2 (±2.1). Using carbogen with a 2% CO2 content the TGT with a PIBT of 5 min. was only at 23.3 days (±1.5), but even after a PIBT of 60 min. there was no apparent drop-off in sensitisation, with the TGT being 25.5 days (±5.4).

Conclusion: These preliminary results suggest that reducing the CO2 content from 5% to 2% can still improve the radiation response of this C3H mouse mammary carcinoma. But, while 2% may not be as effective as 5% with a short PIBT it may actually be suprior with a longer PIBT interval.

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Combination of insulin-like growth factor-1 (IGF-1) and amifostine increases the tolerance of the spinal cord to re-irradiation

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Objective: To test whether IGF-1 and amifostine modulate re-irradiation tolerance of the rat cervical spinal cord. Initial experiments by our group suggested that administration of each agent alone significantly increased median latent time to radiation myelopathy (RM) in previously unirradiated animals but did not change the dose-response relationship. Because of different modes of action, a follow up study was undertaken to test the combined treatment

Methods: The cervical spinal cord of 90 adult Fisher F-344 rats received a single fraction of 16 Gy, which corresponds to approximately 75% of the median paresis dose (ED50), followed five months later by a second radiation dose. Re-irradiation dose ranged from 17 to 25 Gy in the treatment group (n=59) and from 17 to 23 Gy in control animals (n=31). The study animals received a single intrathecal injection of 0.3 mg amifostine into the cisterna magna 30-60 min before re-irradiation plus three subcutaneous doses of IGF-1 (700 mcg) starting from 24 h before to 24 h after re-irradiation. Control animals received saline injections via the same routes. Animals were followed until RM developed or until at least 9 months from re-irradiation. Histopathologic examinations were performed post mortem.

Results: No animals showed any neurologic abnormalities before re-irradiation. RM occurred in 22 control animals after a median latency of 117 days (92-212 days) from second dose. In contrast, only 5 treated rats developed RM (after 108-174 days) within 270 days, $p<0.05$. ED50 was 18.5 Gy (95% confidence interval 17.2-19.6 Gy) in the control group versus 24.6 Gy (22.1-58 Gy) in the treatment group. However, within comparably irradiated groups, i.e. 17-23 Gy, 11 rats receiving IGF-1 plus amifostine (6/11 received 23 Gy) versus none of the control rats died of unknown causes within 30 days after re-irradiation. Gross and histopathologic lesions in these rats that died unexpectedly were insufficient to determine the cause of death.

Conclusion: The experimental data revealed supra-additive effects of IGF-1 and amifostine in reducing radiation neurotoxicity resulting in increasing the ED50 by more than 30%. This finding strengthens the evidence that brief therapeutic intervention can decrease radiation-induced neurotoxicity. However, unexpected from our earlier study in previous unirradiated rats, the regimen also induced mortality in re-irradiation setting. Further studies will be undertaken to optimize the regimen.

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EGF-receptor tyrosine kinase inhibition combined with fractionated radiotherapy in human squamous cell carcinoma xenografts

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Purpose: Proliferation of clonogenic tumour cells during fractionated irradiation is a major cause of local failure in squamous cell carcinoma (SCC). The EGFR signal transduction pathway has been suggested to play an important regulative role in this process. The aim of our study was to investigate whether specific inhibition of the EGFR-TK by BIBX1382BS improves the results of fractionated irradiation of EGFR-positive FaDu hSCC in nude mice.

Methods: Proliferation rate, cell cycle distribution and BrdUrd-LI, and clonogenic cell survival were determined in vitro after application of 5 µmol/l BIBX1382BS or carrier. Tumor-bearing nude mice received BIBX1382BS (50 mg/kg/d) alone or simultaneously with fractionated RT. Experimental endpoint was tumor growth delay. In addition histological investigations on BrdU-LI, Ki67-LI, necrosis and apoptosis were performed.

Results: In line with histological and FCM results showing a decreased BrdUrd labelling and accumulation of cells in G1, BIBX1382BS significantly decreased the growth of FaDu cells in vitro and of FaDu tumors in nude mice. In vitro BIBX1382BS was slightly cytotoxic. When given simultaneously to 15x2 Gy, BIBX1382BS had no effect on tumor growth delay.

Conclusion: EGFR-TK inhibitor BIBX1382BS significantly decreases proliferation of FaDu tumors. Results after a short course of fractionated RT were not improved. However, as repopulation of clonogenic cells in FaDu tumors has been shown to accelerate after 3 weeks of fractionated RT, it appears possibly that combined treatment may be more effective after longer overall treatment times. This question is currently investigated.

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Reduced DNA-dependent protein kinase activity in two cell lines derived from individuals with radionecrosis

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Background: Late normal tissue toxicity limits the dose of radical radiotherapy. In mammalian models, radiosensitivity is almost invariably associated with DNA repair defects. To investigate the role of this phenotype in late radionecrosis, we have examined the activity of enzymes involved in non-homologous endjoining (NHEJ) and double-strand break repair in cell lines derived from patients with late radiation injury.

Aim: To assess the effect of NHEJ enzyme activity on late radiation injury.

Methods: Patients with necrosis (grade 4 or 5 RTOG late morbidity) after radical radiotherapy were identified from the departmental database of patients treated since 1974. Sections from paraffin-fixed archival blocks were stained with antibodies against enzymes involved in NHEJ. EBV-transformed lymphoblastoid cell lines were derived from 5 patients who sustained injury at "safe" doses. Control cell lines were obtained from 3 patients without cancer. Post-radiation viability was assessed by colorimetric absorbance. DNA-dependent protein kinase (DNA-PK) activity was assayed with biotinylated peptide substrate. NHEJ enzyme expression was determined by immunoblotting.

Results: Post-radiation viability in cell lines (LB0003 and LB0004) derived fron two patients with radionecrosis was intermediate between an ataxia-telangiectasia cell line and normal controls. These two cell lines exhibited 8-fold reduction in DNA-PK activity. Sections from a post-radiation cervix biopsy in one patient, and the bilateral breast cancers in the second, showed no evidence of staining with antibodies against DNA-PKcs. NHEJ enzymes were expressed in all cell lines.

Conclusion: These data suggest reduced DNA-PK activity may be implicated in late radiation injury in some patients.

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Combination of the TRAIL death ligand with ionizing radiation - rationale and efficacy

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Rationale: A combination of antitumor approaches acting on different death pathways seems ideal for increasing therapeutic responses, especially when defined resistance mechanisms interfere with individual cellular processes.

Materials and methods: Apoptosis induced by TRAIL or ionzing radiation (XRT) alone or in combination was analyzed by FACS. Caspase-8/-9 and BID activation was analyzed by western blotting. Mitochondiral damage was inhibited by overexpression of Bcl-2

Results: Both TRAIL and XRT induced activation of caspase-8, caspase-3, BID and mitochondrial potential loss. TRAIL induced apoptosis required caspase-8, whereas it was not essential for radiation induced apoptosis. Inhibition of mitochondrial damage by Bcl-2 abrogated XRT induced apoptosis and caspase activation, but attenuated TRAIL induced apoptosis only. Combined treatment TRAIL/XRT exerted additive apoptotic effects in control cells, whereas synergistic effects occurred in cells overexpressing Bcl-2. A strong effect of TRAIL on radiation induced clonogenic cell death was found. Similar data were obtained with solid tumor lines (MCF-7, R30C, Colo 824, BT474 (Breast) A549 (Lung) FaDu, SCC4, SCC9 (H&N) HT29 (Rectum). All lines except Colo 824 were apoptosis resistant when irratiated with 10 Gy. However TRAIL induced cell death in SCC4, R30C, HT 29, A549, BT474, FaDu. No response was detectable in fibroblasts. Preirradiation induced strongly increased TRAIL effects in R30C and SCC4 cells

Conclusion: The TRAIL death ligand seems to be of high potential value for a combination with ionizing radiation in tumor therapy.

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Evidence for the p53 tumour suppressor protein as a direct sensor of DNA damage

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Mammalian cells delay their cell cycle progression after DNA damage (ie. G1 and G2 cell-cycle checkpoints), presumably to allow time for DNA repair, thereby maintaining their genomic integrity. Molecular data exists to suggest that focal DNA repair protein-protein interactions (ie. rad50-mre11; rad51-BRCA1) occur within the nuclei of irradiated cells at sites of DNA-dsb following IR, but whether these focal interactions occur secondary to direct signals and interactions with DNA damage checkpoint sensing protein (ie. p53, ATM) is unknown. Indeed, the wild type p53 G1-checkpoint can be activated with as little as one DNA-dsb and cause a permanent G1 arrest in lethally irradiated fibroblasts. As yet, direct evidence that the p53 protein can sense and activate DNA-dsb repair following irradiation as part of a DNA damage checkpoint response is lacking. To test the hypothesis that the p53 protein can sense DNA breaks in vivo, we have obtained data using quantitative immunofluorescence, confocal microscopy with antibodies to specific phospho-forms of p53. In a dose-responsive manner, normal human fibroblasts irradiated in plateau phase (ie. GM05757) show an accumulation of discrete nuclear foci when stained with an antibody to the serine-15 phosphorylated form of p53 (ie. ser15-p53) which is a form activated by IR in an ATM-dependent manner. Dose-responsive foci can be observed within 30 minutes of IR-exposure, suggesting that p53 rapidly localizes to sites of IR-induced damage. A kinetic study of ser15-p53 accumulation in GM05757 cells suggest that despite a rapid induction of ser15-p53 following IR, a high level of residual foci remain at 24 hours which correlates to the level of rad50 foci. Rad51 foci are not dose-responsive and are