Radiobiology

563 ORAL

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

M. Nordsmark[1], J. Loncaster[2], C. Aquino-Parsons[3], S. Chou/Varia[4], M. Ladekarl[1], C. West/Davidson[2], J. Lindegaard/Havsteen[1], R. Hunter[2], J. Overgaard[1], J. Raleigh[4]. [1] *Aarhus university hospital, Dept. Oncology an dExp. Clin. Oncology, Aarhus, Denmark;* [2] *Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Manchester, England;* [3] *BC Cancer Agency, Dept. Radiation Oncology, Vancouver, Canada;* [4] *UNC-CH School of Medicine., Dept. Radiation Oncology, Chapel Hill, NC, USA*

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).

564 ORAL

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

A. Giatromanolaki[1,2], E. Sivridis[1,2], K.C. Gatter[1,3], A.L. Harris[1,3], M.I. Koukourakis[1,3]. [1] *Tumour and Angiogenesis Research group;* [2] *Democritus University of Thrace, Alexandroupolis,Greece;* [3] *Institute of Molecular Medicine, Oxford,UK*

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.

565 ORAL

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

M. Horsman, J. Overgaard. *Danish Cancer Society, Dept. Experimental Clinical Oncology, Aarhus University Hospital, Aarhus, Denmark*

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.

566 ORAL

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

C. Nieder[1], R.E. Price[3], B. Rivera[3], K.K. Ang[2]. [1] *Klinikum rechts der Isar, TU Munich, Dept. of Radiation Oncology, Munich, Germany;* [2] *University of Texas M.D. Anderson Cancer Center, Dept. of Experimental Radiation Oncology, Houston, TX, USA;* [3] *University of Texas M.D. Anderson Cancer Center, Dept. of Veterinary Medicine and Surgery, Houston, TX, USA*

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.