particles in tissue has so far precluded their use against solid tumors. We have developed a new form of brachytherapy that enables the treatment of solid tumors with alpha radiation, which was termed- Diffusing Alpha-emitters Radiation Therapy (DART). The basic idea of DART is to insert into the tumor sources loaded with ^{224}Ra atoms, which release from their surface short-lived alpha-emitting atoms. These disperse inside the tumor and deliver a lethal dose through their alpha decays. The present study examines the anti-tumoral effects resulting from the release of alpha emitting radioisotopes into solid lung carcinoma (LL/2 and A427) tumors. We assessed the efficacy of the short-lived daughters of ^{224}Ra, which are released into the malignant tissue, to produce tumor growth retardation and prolong life.

Methods: Radioactive wires (0.3 mm diameter and 5 mm long) with ^{224}Ra activities in the range 12-33 kBq were inserted into LL/2 tumors in C57BL/6 mice and into human derived A427 tumors in athymic mice. Tumor development was recorded during 21 days (LL2) or 28 days (A427) and survival was monitored for 45 days (LL2) or 120 days (A427). An in-vitro set-up tested the dose dependent killing of tumors cells exposed to alpha particles.

Results: The insertion of a single DART wire into the center of 6-7 mm (130 mm^3 average volume) tumors had a pronounced retardation effect on tumor growth in the murine model, leading to a significant inhibition of 49% (LL2) and 93% (A427) in tumor development, and prolongations of 48% (LL2) in life expectancy. These observed effects were strengthened when tumors were treated with two DART wires. In the human model more than 80% of the treated tumors disappeared or shrunk. Autoradiographic analysis of the treated sectioned tissue revealed intratumoral distribution of radioactive atoms around the wires, and histological analysis revealed corresponding areas of necrosis. In-vitro experiments demonstrated a dose-dependent killing of tumors cells exposed to alpha particles.

Conclusions: The results indicate that DART causes significant damage to lung carcinoma, and prolongs survival. DART holds great potential for the treatment of human lung cancer, and might be augmented by chemotherapy and other modalities like immunotherapy or anti growth factors.

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Ionizing radiation fosters cancer invasion through transcriptional up-regulation of the MET oncogene

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It is debated whether ionizing radiation (IR) employed in radiotherapy could unleash tumor invasion and metastasis, as suggested by animal models. We have shown that, in epithelial and melanoma cell lines, IR induces overexpression of MET, the tyrosine kinase receptor for hepatocyte growth factor (HGF). MET is an unconventional oncogene that drives "invasive growth", a complex genetic program, which controls tissue development and regeneration in physiological conditions, but can promote metastasis in cancer cells. We have found that MET induction by IR is supported by transcription factors activated in response to DNA damage and extracellular stress stimuli. We have then shown that MET upregulation is crucial for the biological effects elicited by IR. In fact, in vitro IR induces spontaneous cell invasion that requires MET expression and kinase activity. Moreover, IR exposure sensitizes cancer cell lines to HGF, and turns the physiological response to this signal (branching morphogenesis) into disorganized cell spread and migration in tri-dimensional matrices. These findings support the notion that MET upregulation is part of a transcriptional program elicited in response to DNA damage, and likely aimed to promote cell recovery and repair. However, MET expression/activation in irradiated cancer cells can convert a healing response into an invasive program leading to cancer dissemination, thus providing a molecular explanation for the adverse effects of radiotherapy.

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Suppression of DNA-PK effected the radiosensitivity and cell cycle of HeLa

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In response to DNA damage, cell survival can be enhanced by activation of DNA repair mechanisms and of check-points that delay cell cycle progression to allow more time for DNA repair. DNA Double-strand break (DSB) is the most lethal of all DNA lesions and is repaired by two major repair pathways: homologous recombination (HR) and nonhomologous end-joining (NHEJ). It is generally believed that NHEJ plays a more important role than HR in mitotically replicating cells especially in human cells. DNA-dependent protein kinase (DNA-PK), including Ku80, Ku70 and DNA-PK catalytic subunit (DNA-PKcs), is the most important kinase plays in NHEJ. We produced deficiencies of Ku80 by small hairpin interfering RNA (shRNA) in human cervical carcinoma cell line HeLa, before exposure to 6MV X-ray. The shRNA led to a ~96% reduction of Ku80 expression, and made the dose-reduction factor (DRF) of 10% cell survival got to 1.68; Transfection DNA-PKcs-targeted shRNA into HeLa/Ku80-shRNA gave rise to a DRF of 2.47 compared to control cells; while after pretreated with 50μM LY294002, a chemically specific phosphatidylinositol (PI) 3-kinase inhibitor (DNA-PKcs and the major HR protein ATM are both members of PI 3-kinase gene family), HeLa/Ku80-shRNA's DRF of 10% cell survival comparison to control cells even got to 3.36. As for cell cycle distribution after 6Gy X-ray exposure, just like control cells, HeLa deficiency of Ku80 singly or in combination with DNA-PKcs showed the cell accumulation in G2/M phase, and the G2/M accumulation got to vertex at 48h post-irradiation. However, HeLa cells transfected only with DNA-PKcs-targeted shRNA or pretreated with LY294002 had a prolong G2/M delay, the percentage of cells in G2/M phase even didn't get to highest at 72h post-irradiation, it suggests that the accumulation of significant unrepaired DNA damage following inhibition of DSB repair proteins. These data indicates that the role of Ku80 in DSB repair could be compensated by other DSB repair protein after its inhibition, co-inhibition of both NHEJ and HR proteins would be a suitable stratagem to enhance the radiosensitivity of cancer cells; Based of the cell cycle consequences, we presumed that DNA-PKcs and Ku80 had different contributions in cell cycle distribution, Ku80 firstly acted in cell cycle response, and then induced the participation of the DNA-PKcs, when Ku80 protein was inhibited, the HR protein would compensate completely, when DNA-PKcs was suppressed, for the "occupying" of Ku protein, neither HR nor NHEJ could act normally, resulted in the delay of cell cycle.

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Gefitinib enhances radiosensitivity of glioma neurospheres by inhibition of Akt signalling and DNA repair mechanisms

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Background: Brain tumor stem cells (BTSCs) have recently been identified as critical for maintenance of tumor mass, contributing to chemo-radioresistance and tumor relapses. We investigated the effect of irradiation on stem-like glioma neurospheres and determined whether the tyrosine kinase inhibitor gefitinib could enhance radiosensitivity by inhibition of Akt activation and DNA repair mechanisms. Methods: Malignant glioma tissues were collected with informed consents from patients in accordance with protocols approved by the institutional review boards at National Cancer Centre, Singapore. Tissues were minced, trypsinised and cultured for growth of neurospheres and differentiated glioma cells. Neurospheres that exhibited characteristics of BTSCs (self-renewal and multipotent) were used in this study. Dose-dependent effects of irradiation (0-10 Gy) on neurospheres and differentiated glioma cells were analysed for clonogenic formation (14 days), gamma-H_2AX (marker for double-strand DNA breaks) immunostaining and CD133+ (marker for neural stem cells) cell population. Neurospheres and differentiated gliomas treated with gefitinib (5 μM), irradiation (5 Gy) or both were assayed for clonogenic formation, Akt phosphorylation (Ser 473) and DNA-PK complex formation. Results: Irradiation did not affect clonogenic survival and gamma-H_2AX immunostaining of glioma neurospheres, but significantly reduced clonogenic survival and increased gamma-H_2AX immunostaining of differentiated glioma cells in a dose-dependent manner. Irradiation significantly increased CD133+ cell population in neurospheres, but not in differentiated glioma cells. Gefitinib significantly inhibited clonogenic survival of irradiated neurospheres, but not differentiated glioma cells. Akt phosphorylation was inhibited by gefitinib alone and when combined with irradiation in neurospheres, but not in differentiated glioma cells. Irradiation slightly inhibited DNA-PK complex formation in neurospheres, with further inhibition by combined gefitinib and irradiation. In differentiated glioma cells, irradiation enhanced DNA-PK complex formation with no further effect by combined gefitinib and irradiation. Conclusions: Stem-like glioma neurospheres are resistant to irradiation-induced DNA damage, with greater clonogenic survival than irradiated differentiated glioma cells. Gefitinib enhanced radiosensitivity of stem-like glioma neurospheres by inhibition of Akt activation and DNA repair mechanisms.