Akt1 facilitates DNA double-strand breaks repair through a direct physical interaction with DNA-PKcs

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Purpose or Objective: It is well known that PI3K/Akt pathway is hyperactivated in K-RAS mutated tumor cells and is involved in radioresistance. Exposure to ionizing radiation induces activation of DNA-dependent protein kinase catalytic subunit (DNA-PKcs) as an essential enzyme for repair of DNA double-strand breaks (DSBs) through non-homologous end joining. Radiation-induced DNA-PKcs activity is partially dependent on serine/threonine kinase Akt1. In this study, role of DNA-PKcs in Akt1-mediated DSB repair and post-irradiation cell survival was investigated. Likewise, a direct physical interaction of Akt1 with DNA-PKcs was studied.

Material and Methods: Non-small cell lung cancer cell line A549 and colorectal cancer cell line HCT116 with point mutations in K-RAS gene were utilized. Complex formation of Akt1 with DNA-PKcs and role of Akt1 in DSBs repair were tested by immunoprecipitation and γH2AX foci assays, respectively. Localization of Akt1 to DSB site was tested by immunofluorescence staining and confocal microscopy of P-Akt (S473) and γH2AX following microbeam laser irradiation and after exposure to ionizing radiation. To determine the potential interacting domain of Akt1 with DNA-PKcs, GST, GST-Akt1 full-length, GST-Akt1-N-terminal fragment (1-150 a.a.), and GST-Akt1-C-terminal (151-480 a.a.) proteins were incubated with purified DNA-PKcs and pull-down assay was performed. In order to identify the domain of DNA-PKcs that interacts with Akt1, constructs expressing four distinct fragments of DNA-PKcs (1-426, 427-1400, 2401-3850, 3700-4188 a.a.) tagged with EGFP and full length Akt1 tagged with mCherry were produced. Akt1/DNA-PKcs was studied in A549 cells, transiently transfected with the appropriate constructs.

Results: Akt1 formed a complex formation with DNA-PKcs in the nuclear fraction immediately after irradiation. Nuclear Akt1 was co-localized with γH2AX foci and found to be essential for the efficient repair of ionizing radiation-induced DSBs and post-irradiation cell survival, in a DNA-PKcs dependent manner. A direct physical interaction of DNA-PKcs to the C-terminal domain of Akt1 could be demonstrated. Additionally, Akt1 was found to make physical interaction not only with the C-terminal domain of DNA-PKcs (3700-4188 a.a.) but also with the N-terminal domain (1-426 a.a.).

Conclusion: Akt1, through a direct physical interaction with DNA-PKcs, regulates repair of ionizing radiation-induced DSBs. Thus, due to overexpression of Akt1 in tumor cells and constitutive Akt activity in K-RAS mutated tumors cells, Akt1 can be proposed as a tumor specific target for radiosensitization.

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Proffered Papers: Clinical 5: Upper and lower GI

OC-0239 Survival of clinical stage I-III rectal cancer patients: a population-based comparison
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Purpose or Objective: Total mesorectal excision is the cornerstone of rectal cancer treatment and preoperative (chemo)radiotherapy and adjuvant chemotherapy are often administered. This population-based study compares the survival in clinical stage I-III rectal cancer patients who received either preoperative radiotherapy, preoperative chemoradiotherapy or no preoperative therapy. The effect of type of radical resection and adjuvant chemotherapy on survival was also investigated.

Material and Methods: Patients diagnosed between January 2006 and December 2011 with clinical stage I-III rectal adenocarcinoma were retrieved from the national Cancer Registry database. Only first primary invasive rectal tumors were included and only patients who underwent a radical resection were retained. The observed survival was
calculated from the date of surgery until the date of death or until the last known vital status. Conditional survival was defined as the survival conditional on surviving one year after surgery and was calculated in order to avoid the impact of adverse events in the postoperative course. Multivariable Cox proportional-hazards regression models were applied to evaluate the association of preoperative treatment, type of radical resection and use of adjuvant chemotherapy with survival, adjusting for the baseline characteristics age, gender, WHO score and clinical stage.

Results: A total of 5173 eligible rectal cancer patients were identified from the national database. Preoperative treatment was as follows: none in 1354 (26.2%), radiotherapy in 797 (15.4%) and chemoradiotherapy in 3022 (58.4%) patients. Patients who received no preoperative therapy or preoperative radiotherapy and those who underwent abdominoperineal resection had a lower observed survival as compared with patients receiving preoperative chemoradiotherapy or treated with sphincter-sparing surgery respectively (Table). The patient group receiving adjuvant chemotherapy had a worse observed survival than the group receiving no adjuvant therapy. These effects were age-dependent. Multivariable analysis demonstrated similar findings for the observed survival conditional on surviving the first year after surgery.

Conclusion: In this population-based study, preoperative chemoradiotherapy, sphincter-sparing surgery and no adjuvant chemotherapy were associated with a superior survival in clinical stage I-III rectal cancer patients.

OC-0240
Lumbarsacral bone marrow modeling of acute hematological toxicity in chemoradiation for anal cancer
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Purpose or Objective: To model acute hematologic toxicity (HT) and dose to pelvic osseous structures in anal cancer patients treated with definitive chemoradiation (CT-RT).

Material and Methods: 53 patients receiving CT-RT were analyzed. Pelvic bone marrow (PBM) and corresponding subsites were contoured: ilium (IBM), lower pelvis (LPBM) and lumbosacral spine (LSBM). Dose-volume histograms points and mean doses were collected. Logistic regression was performed to correlate dosimetric parameters and >G2-G3 HT as endpoint. Normal tissue complication probability (NTCP) was evaluated with the Lyman-Kutcher-Burman (LKB) model.

Results: Logistic regression showed a significant correlation between LSBM mean dose and >G2 neutropenia (B coefficient:0.109;p=0.037;95%CI:0.006-0.212) and >G3 leukopenia (B coefficient:0.122;p=0.030;95%CI:0.012-0.233) (Table 1). According to NTCP modeling, the predicted HT probability had the following parameters: TD50:32.6 Gy, y50:0.449 (>G2 neutropenia) and TD50:37.5 Gy, y50:1.15, m:0.347 (>G3 leukopenia) (Figure 1). For node positive patients TD50:30.6 Gy, y50:2.20, m:0.181 (>G2 neutropenia) and TD50:35.2 Gy, y50:2.27, m:0.176 (>G3 leukopenia) were found (Figure 1).