perform the selection procedure for a set of patients. Subsequently, the choices are discussed in the group of observers and a set of selection rules is composed. In this lecture we will discuss the plan selection strategy for rectum cancer and its introduction in the clinic.

Poster Viewing : 9: Radiobiology

PV-0424

Cyclin D1 silencing radiosensitises prostate cancer cells by impairing DNA-DSBs repair pathways.

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Purpose or Objective: Patients with hormone-resistant prostate cancer (PCa) have higher biochemical failure rates after radiation therapy. Cyclin D1 deregulated expression in PCa is associated with a more aggressive disease however its role in radioresistance has not been determined.

Material and Methods: Cyclin D1 levels in the AR-negative, androgen-independent PC3 and AR-positive, androgen-independent 22Rv1 cells were stably inhibited by transfection with Cyclin D1-short hairpin RNA (shRNA). Tumorigenicity and radiosensitivity were investigated using *in vitro* and *in vivo* experiments.

Results: Independently by AR-expression, Cyclin D1 silencing interfered with PCa oncogenic phenotype by inducing growth arrest in the G1 phase of cell cycle and reducing soft agar colony formation, migration, invasion, tumor formation and neo-angiogenesis in xenografted mice. *In vitro* colony formation and *in vivo* tumor growth of the PCa xenografts were significantly inhibited by Cyclin D1 silencing combined with radiotherapy. Cyclin D1 silencing radiosensitizes PCa cells by impairing the NHEJ and HR pathways responsible of the DNA double-strand break repair. Cyclin D1 directly interacts with activated-ATM, -DNA-PKC and RAD51 that are downstream targets of Cyclin D1-mediated PCa cells radioresistance.

Conclusion: Taken together, these observations suggest a Cyclin D1 role in radioresistance mechanism. Cyclin D1 could represents a potential target for radioresistent androgen-sensitive or not prostate cancer cells.

PV-0425

EEF2K promotes progression and radioresistance of esophageal squamous cell carcinoma

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Purpose or Objective: We investigated the effects of eukaryotic elongation factor 2 kinase (EEF2K) in esophageal squamous cell carcinoma (ESCC) and its role in radiosensivity.

Material and Methods: We used quantitative real-time polymerase chain reaction and immunohistochemistry analyses to compare expression of EEF2K between paired ESCC samples and nontumor esophageal tissues. Lentivirus was used to overexpress and knockdown of EEF2K gene and stable transmitted cell line of ECA109 and TE13 were made. In vitro cell counting kit 8 and clone formation assay were used to detect cell viability and proliferation. Wound-healing migration assay, transwell invasion assay three-dimensional culture and tube formation assay were used to investigate invasion, metastasis and angiogenesis of ESCC. Radioresponse was primary examined by clone formation assay after exposure of 0, 2, 4, 6, 8 Gy X-ray by a medical accelerator of different stable cell lines. Then apoptosis, cell-cycle arrest, and γ-H2AX expression were examined in 0 Gy and 8 Gy in the overexpressed and knockdown ESCC cell line by flow cytometer and immunoflourence. Gene-chips and western blot were used to investigate molecular mechanism. In vivo experiments of xenografts were used to confirm the results.

Results: Levels of eEF2K were increased 52.17% of ESCC samples compared with matched nontumor tissues, as well as ESCC cell lines. Increased levels of eEF2K were associated with ESCC survival times of patients ($P<0.05$). eEF2K expression correlated between tumor size and TNM stage in primary ESCC during clinicopathological feature analysis ($P<0.05$). EEF2K promotes ESCC proliferation and tumorgeneity in vitro and in vivo. Improved invasion, metastasis and angiogenesis were also seen in EEF2K overexpressed cells compared with control in TE13 and ECA109 cell lines. An improved radioresponse was detected in eEF2K knockdown cells which could also be induced by NH125, an eEF2K inhibitor. Affymetrix GeneChip were used in EEF2K overexpressed ECA109 and control cells in normal conditions and 8 Gy of irradiation and autophagy pathways were detected by bioinformatic analysis. Improved protein expression of Atg5, mTOR, LC3, and TP53 were confirmed by western blot. In xengraft radiosensivity experiments, an enhancement factor of 1.78 was seen in ECA109 bearing nude mouse by NH125, along with a reduction of tumor doubling time. Immunohistochemistry and immunofluorescence of tumor tissue confirmed the molecular mechanism of autophagy pathway.

Conclusion: EEF2K is overexpressed in ESCC and associated with progression and shorter survival times of patients. Decreased expression of EEF2K correlated with a reduction of malignancy in biological behavior and an improvement of radioresistance in ESCC, which may be mediated by autophagy signaling pathway. Targeting EEF2K may be a potential therapeutic approach of ESCC in the future.

PV-0426

Targeting PI4K for radiosensitisation: a viable model of drug repositioning

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Purpose or Objective: Phosphatidylinositol 4-phosphate (PI4P), upstream regulator of both phospholipase C (PLC)/Protein Kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) / serine/threonine-protein kinases (Akt) pathways which control the cell motility and proliferation, is produced by phosphatidylinositol 4-kinase (PI4K). Thus, an inhibition of PI4K could inactivate these two PI4P dependent pathways simultaneously. In this study, we tried to identify that which isotype of PI4K may affect a radiosensitivity using RNA interference (RNAi) and also to investigate anti-hepatitis C viral (HCV) agents which are known to inhibit PI4K activity, could be repositioned as a radiosensitizer in human breast cancer, glioblastoma and hepatoma models.

Material and Methods: A panel of human cancer cell lines including U251 malignant glioma cells, BT474 breast cancer cells, and HepG2 hepatocellular carcinoma cells were used. RNAi was used to specific inhibition of each isotype of PI4K and clonogenic assay was performed to assess the radiosensitizing effect of each isotype. To select an anti-HCV agent for pharmacologic inhibition of PI4K, IC50s of nine commercial antiviral agents were determined. Specific inhibitory effect on PI4K isotype was determined by *in vitro* kinase assay. Radiosensitizing effect of the selected anti-HCV agents were tested by clonogenic assay *in vitro* and tumor xenograft model *in vivo*, respectively. Immunoblotting, immunocytochemistry, and invasion/migration assay were performed to identify the mechanism of radiosensitization.