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 radiosensitivity 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 radiosensitivity.

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

**PV-0425**
EEF2K promotes progression and radiosensitivity 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 radiosensitivity.

**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 *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. Radiosensitivity 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 immunofluorescence. Gene-chips and western blot were used to investigate molecular mechanism. In *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 tumorigenity 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 radiosponse 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 xenograft radiosensitivity 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 radiosensitivity 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), suppresses 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 PI4K 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 hematoma 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.
Results: First, we identified that specific inhibition of PI4K IIla using RNAi increased radiosensitivity in the human cancer cell lines we tested. In contrast, inhibition of other isotypes did not affect a radiosensitivity of these cancer cell lines. Next, in vitro kinase assays showed, simprevir, a selected anti-HCV agent via IC50 assay, inhibited activity of PI4K IIla in a dose-response manner. Pretreatment with simprevir induced discernible downregulation of p-PKC and p-Akt and also increased clonogenic survival of U251, BT474, and HepG2 cells in vitro and also significantly delayed growth of mouse tumor xenografts in vivo. Simprevir caused prolongation of γH2AX foci after irradiation, decreased invasion / migration and downregulation of PD-L1 expression.

Conclusion: Targeting PI4K IIla using anti-HCV agent could be a viable drug repositioning approach to enhance the therapeutic efficacy of radiotherapy for breast cancer, glioblastoma and hepatoma. (Work supported by grant #2013R1A1A2074531 from the Ministry of Science, ICT & Future Planning to Kim IA)

PV-0427
Real-time tumour oxygenation changes following a single high dose radiotherapy in mouse lung cancers
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Purpose or Objective: To investigate serial changes of tumor hypoxia in response to a single high dose irradiation by various clinical and pre-clinical methods in order to propose an optimal fractionation schedule for stereotactic ablative radiotherapy (SABR)

Material and Methods: Syngeneic Lewis lung carcinomas were grown either orthotopically or subcutaneously in C57BL/6 mice and were irradiated with a single dose of 15 Gy to mimic SABR used in the clinic. Serial [18F]-misonidazole (F-MISO) positron emission tomography (PET) imaging, pimonidazole FACS analyses, hypoxia-responsive element (HRE)-driven bioluminescence, and Hoechst 33342 perfusion imaging in C57BL/6 mice were performed before irradiation (d-1), at 6 hours (d0), 2 (d2), and 6 days (d6) after irradiation for both subcutaneous and orthotopic lung tumors. For F-MISO, scan was performed 2 hr after the intravenous injection of F-MISO probe and the tumor-to-brain ratio (TBR) was analyzed.

Results: We observed that hypoxic signals were too low to quantitate for orthotopic tumors by F-MISO PET or HRE-driven bioluminescence imaging. In subcutaneous tumors TBR values were 2.87 ± 0.483 at d-1, 1.67 ± 0.116 at d0, 2.92 ± 0.334 at d2, and 2.13 ± 0.385 at d6, indicating that tumor hypoxia was decreased immediately after irradiation and returned to the pretreatment levels at d2, followed by a slight decrease by d6 post-irradiation. Pimonidazole analysis also revealed similar patterns. By using Hoechst 33342 vascular perfusion dye and CD31 co-immunostaining, we found that there was a rapid and transient vascular collapse, which may have resulted in poor intratumoral perfusion of F-MISO PET tracer or pimonidazole delivered at d0 leading to decreased hypoxic signals at d0 by PET or pimonidazole analyses.

Figure 1

Fig. 1. Temporal changes in tumor hypoxia for subcutaneous tumors by F-MISO PET imaging. (A) Representative PET images demonstrating F-MISO uptake in subcutaneous tumor. Arrows indicate the tumor position. (B) A graph showing TBR values for an individual animal. (C) A graph showing the mean ± s.e.m. of TBR values (n = 5).

Conclusion: We found tumor hypoxia levels to be returned to the pretreatment levels by 2 days after irradiation, hence supporting the current fractionation intervals of SABR being given at least 2 days. Our results also indicate that SABR may produce a rapid but reversible vascular collapse in tumors.

PV-0428
Factor 2.5 radiosensitivity difference determined by ex vivo γH2AX assay in prostate cancer patients
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Purpose or Objective: In previous study we showed that γH2AX assay in ex vivo irradiated tumour samples collected from cancer patients of various types correlates with known differences in radioresponsiveness. In the present study we aimed to apply the assay in a panel of prostate tumour