correspondingly. MiR-1246 modified the influence of P53 to mTOR, which indicated its effect in the mediating DNA damage repair and autophagy.
**Conclusion** MiR-1246 decreased the sensitivity of lung cancer cells to radiation through activation of autophagy by targeting mTOR. It was also been demonstrated the significant crosstalk between DNA damage repair and the ups in autophagy. This special mechanism made miR-1246 a potential therapeutic target to overcome the resistance to radiation in non-small cell lung cancer patients.

## PO-125 ROLE OF NRF2 IN BREAST CANCER STEM CELLS RESISTANCE AND TUMOURRECURRENCE

D Kamble*, S Sitasawad. *National Center for Cell Science, Lab-2, Pune, India*

10.1136/esmoopen-2018-EACR25.649

**Introduction** Advancements in radio/chemo therapies have significantly decreased the mortality rate in breast cancer patients; however, resistance and recurrence of the tumour are major concerns with these therapies. Recurrence after radiation therapy is initiated by a subpopulation that is highly radio resistant, known as cancer stem cells (CSCs). One of the main ways of radio therapy to kill cancer cells is by increasing the cellular oxidative stress. Due to higher metabolic and proliferative rate, cancer cells become well-adapted to intrinsic oxidative stress. CSCs are quiescent and work under low levels of oxidative stress It is assumed that CSCs escape radio therapy by modulating redox homeostasis. Nrf2 pathway is a central cellular pathway that protects against oxidative stress. Nrf2 plays a dual role in cancer protection as well as cancer progression by up-regulating the expression of cytoprotective genes and reducing oxidative stress. In our study, we hypothesised that Nrf2 signalling pathway might be involved in radio resistance and low ROS levels in Breast CSCs (BCSCs).
**Material and methods** MCF-7 and MDA-MB-231 cell lines were used for CSC isolation and CSCs were characterised by $CD44^+/CD24^-/ESA^+$, $ALDH^+$ activity. BCSCs were irradiated with fractionated dose of γ-radiation. Protein and mRNA levels were analysed by western blotting and real time-PCR respectively. Radio resistance was checked by colony formation assays.
**Results and discussions** We observed an increase in the $CD44^+/CD24^-/ESA^+$, $ALDH^+$ population and expression of embryonic stem cell markers after fractionated irradiation. BCSCs and non-BCSCs were isolated as $CD44^+CD24^-ESA^+$ and $CD44^-CD24^+ESA^-$. We examined the ROS levels in BCSCs and non-BCSCs after fractionated irradiation. ROS levels were found to be decreased in BCSCs as compare to non-BCSCs after fractionated dose irradiation. We checked the radio-resistance in irradiated cells via radio-resistance assay. More numbers of radio-resistant colonies were formed in the fractionated dose irradiated BCSCs than non-BCSCs. Expression levels of Nrf2 and its downstream regulators were checked in fractionated dose irradiation and were found to be elevated in BCSCs irradiated with fractionated dose than non-BCSCs.
**Conclusion** BCSCs have low levels of ROS and high levels of antioxidants which may contribute to the radio-resistance of BCSCs. Elucidation of role of Nrf2 in BCSCs resistance and tumour recurrence may provide an effective strategy to overcome radio-resistance.

## PO-126 SURVIVAL PROBABILITY OF HUMAN BREAST CARCINOMA CELLS TO RADIATION TREATMENT: ROLE OF CELL FUSION AND OF A SYNCYTIN1-HOMOLOGOUS PROTEIN

[1]R Chignola*, [1]M Sega, [1]B Molesini, [2]A Baruzzi, [3]S Stella, [3]E Milotti. [1]*University of Verona, Department of Biotechnology, Verona, Italy*; [2]*University of Verona, Department of Medicine, Verona, Italy*; [3]*University of Trieste, Department of Physics, Trieste, Italy*

10.1136/esmoopen-2018-EACR25.650

**Introduction** The success of radiotherapy depends on the ability to inhibit tumour growth, and relapse after therapy is determined by cells that retain their clonogenic potential. The radiation sensitivity of isolated tumour cell clones *in vitro* is routinely determined with clonogenic assays. In solid tumours, however, clonogenic cells are not isolated and we carried out experiments to measure the influences of cell-cell contact on their proliferative potential. To this end we developed a new experimental approach to measure the effects of radiation on tumour cell populations. The observations can be understood with the help of a novel stochastic model with a well-defined biological basis.
**Material and methods** T47D cells (human breast carcinoma) were grown at various concentrations in F(flat)-bottom and V-bottom wells of 96-well culture plates. The spheroid outgrowth method was also used to obtain densely-packed tissue cell cultures. A Gammacell40 irradiator equipped with a $^{137}Cs$ source was used to treat cell cultures. Cell fusion was assessed by confocal microscopy. Syncytin 1 expression was assessed by RT-PCR and by flow cytometry using an anti-HERV antibody (clone ab7115, Abcam).
**Results and discussions** The probability of cell survival after 8 Gy radiation treatment increased ~4.7 times when the cells were grown in V-bottom wells as compared to cells grown in F-bottom wells ($p(survival)$=0.0113 and 0.0024, respectively). Microscopic inspections of tissue-like cultures showed that after treatment cell populations were mostly composed of giant cells with multiple nuclei. Cytoplasmic bridges joining different cells were clearly visible. Giant cells and cytoplasmic bridges disappeared at later times (>600 hours) when the cells displayed normal morphology and started to proliferate again. Sequence analysis of cloned RT-PCR products showed that cells expressed a Syncytin 1 homologous protein (Sp). Flow cytometry assays confirmed cytoplasmic expression of Sp and revealed that Sp translocated to the cell surface of irradiated cells committed to death. The fraction of cells surviving 8 Gy treatment was significantly reduced in cultures treated with anti-Sp antibodies.
**Conclusion** Our experimental findings indicate that recovery of breast tumours from radiation is very likely to involve complex pathways that act at the cell population level and that include events of cell fusion mediated by a protein homologous to Syncytin 1.

## PO-127 INVESTIGATING THE RESPONSE OF NORMAL AND CANCER BLADDER CELLS TO RADIOTHERAPY

[1]H Bahmad*, [2]L Bodgi, [1]K Cheaito, [1]T Araji, [2]J Choboq, [2]T Eid, [2]Y Zeidan, [2]F Geara, [1]W Abou-Kheir. [1]*American University of Beirut, Department of Anatomy- Cell Biology- and Physiological Sciences- Faculty of Medicine, Beirut, Lebanon*; [2]*American University of Beirut Medical Center, Department of Radiation Oncology, Beirut, Lebanon*

10.1136/esmoopen-2018-EACR25.651

**Introduction** Bladder cancer is the fourth most commonly diagnosed cancer among males worldwide. Recent advances in