Pancreatic cancer is radiation resistant and this limits the effectiveness of radiotherapy. Incorporation of drugs that radiosensitize pancreatic cancer may increase the value of radiation therapy for pancreatic cancer and lead to better local control. Metformin (N', N'-dimethylbiguanide) is prescribed for managing hyperglycemia. Recent evidence indicates that metformin has antioxidant and tumor growth inhibition activities. To date, no study has shown metformin induces radiosensitization in pancreatic cancer, however radiosensitization has been reported in breast cancer.

**Objective**

To evaluate metformin radiosensitization potential in pancreatic cancer cells.

**Mechanism of action**

Potential mechanism of metformin action on cellular metabolism and cell proliferation. Metformin increases glucose uptake and glycolysis. It activates AMPK leading to inhibition of mTOR. Metformin also inhibits the mitochondrial complex 1. Finally, metformin affects cell growth: it induces p53-dependent autophagy, decrease in protein synthesis, and induces cell cycle arrest through a decrease in cyclin D1 protein level.

**Materials and method**

- Pancreatic cell lines MIA PaCa-2, BxPC-3 and Panc-1 were studied
- Clonogenic and tumor sphere assay to evaluate radiosensitization
- Gamma-H2A.x to study changes in the induction/repair of foci
- FACS to evaluate any cell cycle arrest
- Western blot to study changes in AMPK and LKB1

**Introduction**

- Metformin radiosensitises pancreatic cancer cells and preferentially sensitizes CSCs.
- Radiation plus metformin increases induction of gamma-H2A.x foci
- Combination of metformin and radiation does not induce growth arrest in pancreatic cancer cells
- P'-LKB1 and P'-p70S6K is unchanged in MIA PaCa-2, however, in BxPC3, it increases with metformin and radiation. P'-AMPK is unaltered in both BxPC-3 and MIA PaCa-2
- Compound-C abrogates metformin induce radiosensitization in MIA PaCa-2

**Results**

**Colony formation assay**

**Evaluating cancer-stem-cell-like-cells (CSCC)**

**Assessing DNA damage**

**Cell Cycle analysis**

- MIA PaCa-2, BxPC-3 and Panc-1 cells were incubated with metformin (0, 10, 30 and 100uM) an hour before ionizing radiation (IR) and the clonogenic survival was determined. Colonies were allowed to form in media containing metformin of respective treatment concentration for 10 days. (D) MIA PaCa-2 cells were plated for sphere formation in methocult (MTC) media containing (30uM) of metformin and quantified after 10 days. All data points are means of 3 independent experiments.

**Western-Blot to assess AMPK, LKB-1 and p70S6K**

**AMPK activity necessary for radiosensitization**

**Conclusion**

- Metformin radiosensitises pancreatic cancer cells and preferentially sensitizes CSCs.
- Radiation plus metformin increases induction of gamma-H2A.x foci
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- P'-LKB1 and P'-p70S6K is unchanged in MIA PaCa-2, however, in BxPC3, it increases with metformin and radiation. P'-AMPK is unaltered in both BxPC-3 and MIA PaCa-2
- Compound-C abrogates metformin induce radiosensitization in MIA PaCa-2