SUMMARY AND CONCLUSION:

Earlier studies have shown that the cytotoxicity as well as radio- and chemosensitization induced by 2-DG is heterogenous among various cancer cell lines, which were of different origins varying in their biologic behavior and p53 status (Dwarakanath, 2009; Review). p53 is a critical protein involved in cellular responses to stress (including DNA damage) and is also a key regulator of cell metabolism modulating the balance between the glycolytic and mitochondrial respiratory pathways. Therefore, it was considered worthwhile to investigate the cytotoxic and radiosensitizing effects in isogenic cell lines which vary only in their p53 status. A brief summary of the findings of studies carried out to fulfill this objective in a set of isogenic cell lines of human carcinoma (KB) is presented here. The two mutant cells carried mutations at positions; (a) 68, proline rich region (KB68) and (b) 110, DNA binding domain of p53 (KB110).

Both the mutant cell lines (KB68 and KB110) were found to be more glycolytic (30-40 % increase in glucose utilization) as compared to the wild type cells (KB) and the clonogenic (plating) efficiency of the mutant cell lines (33% and 26%) was lower than the wild type cells (50%).

Cytotoxicity studied by analyzing metabolic viability, clonogenic survival and growth inhibition showed that the effects were dependent on the concentration as well as the duration of exposure to 2-DG in all the cell types. However, KB68 and KB110 cells were more sensitive to 2-DG as compared to KBwt. A significantly enhanced level of 2-DG induced apoptotic cell death was evident in both the mutant cells viz. KB 68 and KB110 cells, suggesting that induction of apoptosis is mainly responsible for the enhanced cytotoxicity.

The oxidative stress induced by 2-DG was relatively more pronounced in both the mutant cells as seen by the higher NADP+/NADPH ratio and ROS levels, suggesting that the enhanced cytotoxicity of 2-DG in KB68 and KB110 was mainly due to enhanced oxidative stress which may be due to alterations in the redox balance possibly due to disturbed balance in the p53 mediated regulation of mitochondrial function (including ROS generation) and glycolysis linked anti-oxidant defense.
Cytotoxicity of 2-DG was also studied in mouse embryonic fibroblasts (MEF) cell lines with p53 knock out p53 (-/-) and Bax knock out Bax(-/-) status by analyzing the metabolic viability. Concentration dependent cytotoxicity was observed in these cell lines; however cytotoxicity was higher in Wt cells compared to p53-/- and bax-/- cells.

Radio-sensitizing effects of 2-DG was found to be heterogeneous among the three cell lines investigated viz. KBwt, KB68 and KB110. A combination of 2-DG (5 mM) and radiation (5Gy) showed an additive effect in the wild type cells (KB), while a sub-additive effect was noted in the KB68 and KB 110 cells. 2-DG showed a marginal effect on the radiation induced cell cycle perturbation in KBWt and KB68 cells, while it induced a significant apoptosis in irradiated KB110 cells.

2-DG enhanced the radiation-induced micronuclei formation to a similar extent in all three cell types (wt, 68 and 110), suggesting that enhanced mitotic death is partly responsible for the radiosensitization and had minimal contribution in the differential effects.

Endogenous ROS was notably higher in both the p53 mutant cell lines (KB68 and KB110) and treatment with the combination (i.e. 2-DG and radiation) further enhanced ROS production in all the three cell lines. However, the accumulation of ROS was significantly higher in KB68 and KB110 cells, which suggests that mutations in p53 status, glucose deprivation (induced by 2-DG) and irradiation all play roles in the enhanced oxidative stress of KB110 and KB68 cells following the combined treatment. Table 5.1 gives an overview of the cytotoxicity and radiosensitizing effects of 2-DG observed in KBWt, KB68 and KB 110 cell lines, while figure 5.1 summarizes the contributions of different pathways responsible for the differential effects of 2-DG seen in wild type and mutant cell lines.
Table 5.1 Overview of the cytotoxic and radiosensitizing effects of 2-DG observed in wild type and mutant cell lines of human carcinoma (KB) cells

<table>
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<th>Parameters</th>
<th>Decrease in metabolic viability</th>
<th>Decrease in clonogenic survival</th>
<th>Induction of apoptosis</th>
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Figure 5.1: A schematic diagram showing the possible reasons for differential cytotoxic and radio-sensitizing effects of 2-DG in tumor cells with wild type and mutant p53.
Following conclusions can be drawn from the results of the present studies:

- Cytotoxicity and radio-sensitizing effects of 2-DG appear to be influenced by the p53 status of the tumor cells (especially head and neck carcinoma cells) possibly due to enhanced metabolic oxidative stress, besides inhibition of repair processes.

- Information on the p53 status in tumors and measurement of glucose usage (using FDG-PET) can help in identification of tumors that would respond better to therapies using 2-DG as adjuvant thereby facilitating the individualization of the therapy.