# SYNOPSIS

**Title of Ph. D. thesis:** Analysis of Interferonγ-mediated Cell Cycle Arrest in Human Cancer Cells – Re-examination of the involvement of Cyclin Dependent Kinase 2

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## Background Information

Interferon gamma (IFNγ) is a potent growth inhibitory cytokine It binds to a specific receptor on target cell surface and triggers a signal transduction pathway through which changes in the expression of specific gene systems are effected. Although many studies have shown the growth inhibitory effect of IFNγ, its mechanism of action remains poorly understood. In the present study different human cell lines were used in order to gain information on the nature of IFNγ-mediated inhibition of proliferation

Earlier studies from this laboratory using unsynchronized cultures of WISH cell line showed that IFNγ inhibits growth by effecting cell cycle arrest at G1/S boundary In the present study, the molecular details of the nature of cell cycle arrest in asynchronous WISH cells were worked out Further, experiments were performed with synchronized population of WISH cells to characterize the action of IFNγ in greater detail Parallel studies were done using the primary colon carcinoma cell line, SW480, and its metastasized form, SW620 to examine their response to the IFNγ-mediated growth inhibition. The study was undertaken with the objective to gain insight into the molecular characteristics of the response of the non-transformed fetal epithelial cell line WISH and the colon cancer cell lines SW620 and SW480 to the growth-inhibitory action of IFNγ

**Summary**

IFN gamma (IFNγ) brings about inhibition of growth of asynchronous WISH cells in a reversible manner detaining the cells at the G1/S boundary Treatment of cells with the cytokine for a duration of 72 hrs led to a severe reduction in the protein as well as activity level of cyclin dependent kinase 2 (CDK2), the kinase that regulates the G1/S transition in the cell cycle Detailed analyses of the factors contributing to this reduced CDK2 activity revealed an overall reduction in the protein and/or activity of majority of the regulators of CDK2 The protein level of the cyclin partner for CDK2 during the G1/S transition, Cyclin E was found to reduce drastically by the end of 72 hrs of treatment with the IFN In addition, the CDC25A phosphatase that removes the inactivating phosphates from CDK2 molecule was also reduced in its protein levels This reduction was reflected in its activity as well, ascertained by an in vitro phosphatase assay for untreated and IFNγ-treated WISH cells

Treatment of asynchronous WISH cells with IFNγ did not affect the protein levels of the kinase present in CDK2 activating kinase CAK II, namely CDK7 but led to severely reduced levels of its cyclin partner Cyclin H and therefore possibly in the kinase activity of CAK II as well The low CDK2 activity in IFNγ-treated WISH cells was not found to be associated with a concomitant increase in the levels of its inhibitory molecules p21 and p27, which also showed reduced protein levels Thus, both the class of regulators of CDK2 activity, the activators as well as the inhibitors were inhibited upon treatment of asynchronous WISH cells with the IFN

The effect of IFN treatment was also perceived by other cell cycle proteins not directly involved in regulating the activity of CDK2 such as the Cyclin D/CDK4 complex Protein levels of Cyclin D were reduced to barely detectable levels by the end of 72 hrs of IFN treatment. This would possibly lead to a reduction in the CDK4 kinase activity although CDK4 protein level remained unaffected The retinoblastoma protein pRb, another regulator of the G1/S transition, showed overall reduction in its protein levels without any preferential increase in its hypophosphorylated active form. The reduced CDK2

activity was therefore a result of the down regulation of most its regulatory molecules The inhibition of other cell cycle proteins was a result of their turnover during the cell cycle arrest of WISH cells upon treatment with the IFN

The nature of proliferation arrest in WISH cells was studied in greater details to ascertain the confinement of the action point of IFN to any specific phase in the cell cycle The question was addressed by treating synchronized population of WISH cells with IFNγ Two synchronization agents, IFNα and Mimosine, with entirely different modes of action, were used to synchronize WISH cells and the reversibility of arrest as also the re-entry of the cells into the cell cycle without any lag was confirmed Cell proliferation assays with IFNα- or Mimosine- synchronized WISH cells treated with IFNγ confirmed that the action point of IFNγ lay within the G1 phase of the cell cycle Molecular studies of synchronized WISH cells treated with IFNγ revealed that the inhibition of synchronized WISH cells did not involve the down regulation of either the protein or the activity of CDK2 Also, neither the cyclin partner of CDK2 at the G1/S, namely Cyclin E, nor the CDK2 activating phosphatase was affected at the level of expression or activity Contrary to the observation with asynchronous WISH population, IFN treatment of synchronized WISH cells lead to an increase in CDK2 activity, indicative of a role for CDK2 in IFN-mediated cell cycle arrest in WISH cells

Parallel studies were carried out using colon carcinoma cell lines SW620 and SW480 to study their response to the antiproliferative action of IFNγ The metastasized SW620 cell line responded to the growth inhibitory action of the IFN in a concentration-dependent manner SW620 cells were resistant to higher concentrations of the cytokine (200 U and above), showing a stimulatory effect on growth with increase in concentration of the IFN IFNγ at concentrations 150 U and below was found to growth inhibit the SW620 cells At the molecular level, the IFNγ-mediated inhibition of growth of SW620 cells was not characterized by reduction in the activity of CDK2, which was similar to the profile of CDK2 in synchronized WISH cells exposed to the cytokine. Other regulatory molecules of CDK2 such as Cyclin E or CDC25A were also not

affected by IFN treatment of SW620 cells at low concentrations Comparative molecular studies with SW620 cells treated with high concentrations of the IFN showed absence of any down regulation of CDK2 activity On the contrary, CDK2 activity was found to increase upon treatment of SW620 cells with the non-inhibitory concentration of the cytokine Response of other cell cycle proteins studied did not reveal any specific pattern to indicate the reason behind inhibition of growth of SW620 cell with low IFN concentration and the stimulation of growth of SW620 cells with high concentrations of the cytokine

SW480, the primary colon cancer cell line continued to remain resistant to the growth inhibitory action of IFNγ irrespective of the concentration of the cytokine used There was no effect on the activity of CDK2 in IFNγ-treated SW480 cells Detailed study of the various cell cycle molecules was not found to indicate any specific reason behind the resistance of SW480 cells to the growth inhibitory action of IFNγ