Chapter 2

Scope for the work
2.1 Introduction

The medical need for advances in cancer treatment with surgery, radiotherapy and conventional cytotoxic chemotherapy, have made only a modest overall impact on mortality. Hence, the significance of discovering new targets, pathways and strategies for therapeutic intervention in cancer is obvious objective. With the emerging observations regarding cell cycle mediated drug resistance, development of optimal schedules and drug combinations require particularly careful attention so that therapeutic potential can be fully realized (Workman and Kaye, 2002). Moreover, the concept of combining chemotherapeutic agents to increase cytotoxic efficacy has evolved greatly over the past several years. The rationale for combination chemotherapy has centered on attacking different biochemical targets, overcoming drug resistance and increasing dose density. The overall goal is to improve clinical efficacy with acceptable clinical toxicity. Any therapeutic strategy aimed at specifically triggering apoptosis in cancer cells has potential therapeutic effect. The fundamental process of progression through cell cycle and of programmed cell death or apoptosis involve the complex interaction of several protein families in systematic and coordinated manner to play role in sensitizing tumor cells to chemotherapy (Shah and Schwartz, 2001). Thus, to amplify the therapeutic effect of cancer chemotherapy, the assessment of molecular mechanisms and targeting apoptosis-related genes may lead to new strategies for the enhancement of ant tumor effect. Identification of oncogenes and tumor suppressor genes has provided molecular links in apoptosis and has boosted enormous interest in the process (Ferreira et al., 2002).

Tumor development is attributed either to p53 deletion, p53 mutation or aberrant p53 function (Hall and Lane, 1997). p53 is involved in distinct functions at the cellular level, namely regulation of normal cell growth and division, gene transcription, DNA repair and genomic stability. Hence, p53 is regarded as a crucial regulatory protein that integrates an array of signals in response to which it turns on a host of biochemical responses at the level of single cell and ultimately the whole organism. p53 activation, resulting in cell cycle arrest or apoptosis would prevent the perpetuation of the genetic defects that would otherwise go unrecognized, multiplying and gradually replacing the normal cell population (Lane, 1992). p53
plays a pivotal role in acting as the mediator between stressful stimuli and the final cellular outcome.

The most frequent alteration in human malignancies is deletion or mutation of the p53 gene and it is the most commonly altered oncogene in development of sporadic and hereditary breast cancers (Ozbun and Butel, 1995). p53 protein overexpression or mutation is rare in normal breast or in benign breast conditions. While sporadic mutations in p53 occur in breast cancers, abnormalities in p53 function appear to be more common than specific p53 gene mutations, unlike many other cancer types. Loss of p53 normal function as the result of loss of heterozygosity (loss of one allele) is more common than the homozygous deletion (loss of both alleles) and is seen in up to 61% of the primary breast cancers (Thompson et al., 1992) and may precede the invasive phenotype (Radford et al., 1993).

2.2 The Rationale

A number of biological and biochemical functions have been ascribed to wild type p53. It is sequence specific transcription factor for cell cycle or apoptosis related genes such as p21, GADD45, Bax, MDM2, Fas, Apaf-1 etc. p53 is a flexible molecule, whose activities are modulated by alterations in its conformation, phosphorylation, acetylation status or localization in the cell. A relevant physiological status of endogenous p53 gene in cancer cells is a key determinant in the outcome of cancer therapy by various cancer chemotherapeutic agents, for regulating cell growth and is also indispensable for the cytotoxic effects (Selivanova and Wiman, 1995; Shen and White, 2001). Activation of the tumor suppressor p53 after cytotoxic insults by chemotherapy or increase in endogenous transactivity may result in two different responses: growth arrest or apoptosis, which may enhance the cell killing in response to chemotherapy. The role of the p53-induced apoptosis in the modulation of the cytotoxicity of anticancer agents, supported the possibility of p53 being a mediator of broad chemosensitivity. The fact that apoptosis is genetically defined pathway, has led to two principle expectations: (a) that the genotype of the tumor will be predictive of the outcome of current cancer therapy; and (b) that new therapies based on apoptosis/cell killing will be superior to present
day anticancer treatments (Brown and Wouters, 1999). The induction of apoptosis, or programmed cell death, in cancer cells is thought to be fundamental to the success of treatments for cancer. On the other hand inactivation of p53 may result in different cascades of signal transduction, which again sensitizes cells to chemotherapy induced cell death via apoptosis. These observations may thus provide implications onto the integration of chemotherapy and p53-responsive 'chemosensitivity genes' (Sionov and Haupt, 1999). Moreover, delineation of the role that p53 may play in cell cycle and anticancer chemosensitization has been hampered by the lack of appropriate model. Different cell lines, experimental protocols, cell growth states or genetic backgrounds may have contributed to the conflicting conclusions. Hence, there is a continuing need for genetically matched cell systems that only differ in p53 protein status to model cellular behaviors that are frequently observed in cancers in relation to both growth and anticancer drug responses. Elucidation of the role of tumor suppressor p53 by its depletion is pivotal to rational understanding of molecular mechanisms implicated in cell growth and chemoresistance in breast carcinogenesis.

One of the emerging strategies against cancer is combination chemotherapy along with searching out effector molecules involved in the cell killing mechanism, in correlation with p53 tumor suppressor status (wild type, mutated or null) of cell types involved. There are three principle reasons to believe that combinations of targeted therapies rather than single agents may yield the most favorable effects. The first is the generic reason: drugs that do not overlap with toxicities or resistance mechanisms may yield additive effects and quantum leaps in benefits. This has certainly been observed with chemotherapy in hematological malignancies and to a lesser extent with breast cancer (Early Breast Cancer Trialists' Collaborative Group, 1998). Second, mathematical synergy using in vitro or in vivo models can be shown by combining targeted agents with chemotherapy or other biological drugs. The third reason is independent of synergy, but is due to the fact that solid tumors display multiple genetic abnormalities and significant heterogeneity. It is unlikely that a single targeted drug would be successful in majority of cases, because of differences in uptake, metabolism, onset or duration of action, pharmacokinetic
behavior, and the mechanism of action of different drugs, simultaneous exposure with multiple drugs may yield a more desirable therapeutic result than sequential order of drug exposure. Furthermore, insufficient cell kill provides ample opportunity for the emergence of resistance. It is becoming clear that large clinical trials will not be feasible to test every combination hence the significance of cell based assays (Tripathy, 2005). Additionally, the major drawback with cancer chemotherapy is the development of chemoresistant cells within the tumors due to their heterogeneous nature, most importantly in the response to therapy under different treatment regimens, and due to inadequate drug delivery methods. It is now widely admitted that breast carcinoma is a genetically and clinically heterogeneous disease hence, the propagation of injury ("bystander effect") from directly damaged cells to other cells has been observed as of great implication in breast cancer therapies. The bystander effect (BE) is a chemo and radiobiological phenomenon that has come to the force recently. It describes the ability of cells affected by an agent to convey manifestations of damage to other cells neither directly targeted by the agent nor necessarily susceptible to it per se. Experiments suggest that this phenomenon is detected in radiation therapy as well as gene therapy in conjunction with chemotherapy. The general advantage of the bystander cell killing phenomenon is the large therapeutic index that can be achieved (Djordjevic, 2000; Hall, 2003).

In summary, considerable scope exists for a detailed investigation on the role of tumor suppressor p53 in normal growth of breast cancer cells as well as in chemotherapy induced apoptosis in these cells, both in vitro and in vivo. Scope for designing strategies to enhance therapeutic potential in breast cancer chemotherapy via p53 modulated signaling is also needed to be explored.

2.3 Aims and Objectives

Since p53 is involved in control of the cell cycle, in repair after DNA damage and in apoptosis, there is a strong biological rationale for investigating the role of p53 as a predictor of response in enhanced therapeutic potential in combination chemotherapy and propagation of injury between cells. We also intend to explore the consequences of inactivation of p53 for elucidation of the role of tumor suppressor p53 in the molecular mechanisms involved in cell growth and...
chemosensitivity in breast carcinogenesis. These investigations will shed light on comprehensive understanding towards future prospects of chemotherapy induced tumor cell killing and the critical role of tumor suppressor gene p53 in breast carcinomas.

The present study was undertaken with following aims and objectives in mind.

**Aims and objectives:**

The proposed study has been accomplished after (A) Developing an isogenic breast cancer cell line differing in p53 status. This will be utilized as a model to investigate the functional consequences of absence of p53 protein in breast cancers. (B) Investigating the effect and mechanism of cytotoxicity of combination of anticancer chemotherapeutic agents, cyclophosphamide and doxycycline on MCF-7 breast cancer cells both in vitro and in vivo and decipher the underlying role of tumor suppressor p53. (C) Developing EGFP expressing MCF-7 and MDA-MB-231 stable cell lines (MCF-EGFP and MDA-MB-231-EGFP respectively) for investigating chemotherapy induced bystander cell killing in breast cancer cells.


2.4 References

