Chapter 4

Regulation of caspase-3 gene expression in MCF-7 cells by doxorubicin
4.1 Introduction

The tumor suppressor p53 plays a major role in regulating the cellular response to various stresses and in maintenance of genomic integrity. Mutations in p53 gene have been detected in more than 50% of human cancers (Soussi and Beroud, 2001). p53 exists in latent state in normal cells and its turnover rate is high due to ubiquitination and proteasome-mediated degradation. However, various stimuli lead to its activation, primarily by protein interaction and post-translational modifications, as discussed in sections 1.4.1 and 1.4.2 of chapter 1. Primary response mediated by p53 upon activation is induction of cell cycle arrest and apoptosis. Both these functions play important role in tumor suppression mediated by p53. Activated p53 drives apoptotic program by transcriptional activation of several target genes that function in induction and execution of apoptosis and also by interacting with Bcl-2, Bcl-XL, Bax and Bak after mitochondrial translocation (Michalak et al., 2005; Erster and Moll, 2005).

p53 activates transcription of its target genes by binding to response elements in the gene. This leads to change in the chromatin structure and transcription initiation by recruitment of proteins involved in transcription (An et al., 2004). The response element that p53 binds to consists of two half sites corresponding to the consensus sequence PuPuPuC(A/T)(A/T)GPypypyp separated by 0-13 bases (Pu stands for purines and Py for pyrimidines) (ei-Deiry et al., 1992). Several proapoptotic members of Bcl-2 protein family like Noxa, Puma, Bax and others are transcriptionally regulated by p53. Expression of Apaf-1, a component of apoptosome that mediates caspase-9 activation and PIDD, a protein implicated in caspase-2 activation via a macromolecular complex termed PIDDosome are transcriptionally regulated by p53. SIVA, a death domain protein, and DR4, DR5 and Fas death receptors are also targets of p53. IGF-BP3, anti-mitogenic IGF signalling inhibitor, is also regulated by p53 and plays a role in cell survival. A large number of p53 target genes have been identified and many of them participate in regulation of cell cycle, apoptosis or cell survival (Michalak et al., 2005).

Apoptosis induced by p53 involves release of mitochondrial intermembrane protein cytochrome c and apoptosome mediated caspase-9 activation (Schuler et al., 2000). Activated caspase-9 in turn activates other down stream caspases, caspase-3 and caspase-7, which will in turn activate caspase-6. Caspase-3 plays a major role in apoptosis as it cleaves PARP, a protein involved in DNA repair and ICAD, an inhibitor of caspase activated DNase (Degterev et al., 2003).
Two other proteins that are members of p53 family, p63 and p73, can also transactivate several p53 target genes (Levrero et al., 2000). Studies using knockout mice have revealed that p63 is involved in epithelial differentiation and limb development while p73 function is important in pheromone detection, female reproduction and survival of Cajal-Retzius neurons in the cortical marginal zone and hippocampus (Mills et al., 1999; Yang et al., 1999; Yang et al., 2000). Although these proteins are similar to p53, no study till date has proved conclusively their function in tumor suppression. A recent study has shown that p63 or p73 function is necessary for proper p53 mediated transactivation of target genes (Flores et al., 2002). Analysis of genes upregulated by overexpression of p73α isoform and p53 identified several genes that are common targets of both the proteins as well as genes that are differentially regulated by them (Fontemaggi et al., 2002). This difference in activation of target genes is likely to be due to difference in the recognition of response elements by p53 family of proteins (Osada et al., 2005).

p73 is activated by DNA damage and upregulates its target genes resulting in apoptosis (Gong et al., 1999). It can induce transcription of Bax gene and lead to Puma-mediated mitochondrial translocation of Bax leading to apoptosis (Melino et al. 2004). Overexpression of p73 leads to ER stress and transcriptional upregulation of Scotin, a protein localized to endoplasmic reticulum (ER) that regulates ER stress induced apoptosis (Terrinoni et al., 2004). Identification of a putative p73 binding site in promoter of Fas gene and upregulation of Fas transcripts by p73 has suggested a possible role for death receptor pathway in p73-induced apoptosis (Ramadan et al., 2005). Recent work from our laboratory has shown that p73 functions in IFNy mediated activation of caspase-1 gene expression (Jain et al., 2005).

Primary function of most of the members of caspase family is in initiation and execution of apoptosis. Activation of caspases occurs by proteolytic processing: autoproteolysis facilitated by protein interactions in the case of initiator caspases or proteolysis by other caspases for downstream caspases. Activated caspases cleave other proteins leading to their inactivation or activation leading to apoptosis. Activation and function of caspases are tightly regulated in cells and plays a role in the outcome of cell survival or death (Danial and Korsmeyer, 2004).

Regulation of caspase gene expression in response to diverse stimuli has been well studied. Several stimuli that initiate apoptotic signalling also regulate caspase gene expression. Moreover, changes in caspase gene expression levels have been detected
in several human disorders and in mouse models. In neurodegenerative conditions such as traumatic brain injury (Ringger et al., 2004), multiple sclerosis (Huang et al., 2004), Huntington's disease (Chen et al., 2000), and Alzheimer's disease (Pompl et al., 2003) and also in other disorders like retinopathy (Grimm et al., 2000) and lymphadenitis (Ohshima et al., 2004), transcript levels of several caspases are found increased. Generally, decreased expression of caspases is associated with resistance to cell death in cancers and enhanced caspase expression correlates with enhanced cell death. Thus levels of caspase gene expression might play a role in deciding the fate of the cells under pathological conditions (Grotzer et al., 2000; Kania et al., 2003; Jee et al., 2005).

Several transcription factors have been implicated in caspase gene regulation. ETS (Liedtke et al., 2003; Liu et al., 2002; Pei et al., 2005), E2F1 (Cao et al., 2004; Pediconi et al., 2003), p53 (Gupta et al., 2001; MacLachlan and El-Deiry, 2002; Liedtke et al., 2003; Rikhof et al., 2003), p73 (Jain et al., 2005) STAT1 (Chin et al., 1997; Kumar et al., 1997), IRF-1 (Tamura et al., 1995) and similar proteins might play a role in regulating caspase gene expression under specific conditions. Sp1 has been shown to play a role in basal level gene expression of different caspases (Liedtke et al., 2003; Liu et al., 2002).

Caspase-1 was the first member of the family that was shown to be regulated by p53 (Gupta et al. 2001). Human caspase-1 promoter has a p53 response element. p53 binds to an oligonucleotide corresponding to this region in electrophoretic mobility shift assays. Overexpression of p53 as well as its activation by various cytotoxic drugs or PTP-S2 overexpression leads to upregulation of caspase-1 mRNA. Inhibition of caspase-1 inhibits apoptosis induced by doxorubicin, PTP-S2 and p53 (Gupta et al. 2001; Gupta et al. 2002).

Doxorubicin is a chemotherapeutic drug that induces double strand DNA breaks leading to p53 activation (Gewirtz et al., 1999). In order to study regulation of caspase gene expression by p53 we analysed levels of different caspase mRNAs in doxorubicin treated MCF-7 cells.

### 4.2 Results

#### 4.2.1 Doxorubicin enhances caspase-3 gene expression in a p53 dependent manner

In order to look at the effect of p53 activation on expression of different caspase genes, RNA was isolated from MCF7 cells treated with 500 ng/ml doxorubicin for 12, 24
Figure 4.1. Effect of Doxorubicin and p53 overexpression on caspase gene expression. (a) MCF-7 cells or MCF-7-mp53 cells, as indicated, were treated with doxorubicin for indicated periods and RNA was isolated. RT-PCR was done for the caspase-3 and GAPDH for required number of cycles and the amplicons were observed on agarose gels. (b) RT-PCR was done to analyse mRNA levels of different caspases in MCF-7 cells after doxorubicin treatment for indicated durations. (c) MCF-7 cells were transfected with pCB6 control plasmid or p53 expression plasmid and RNA was isolated after 24 hours. RT-PCR was carried out to analyse mRNA levels of caspase-3 and GAPDH.
and 48 hours and RT-PCR was carried out for various caspases and GAPDH. Treatment of MCF-7 cells with doxorubicin enhanced expression levels of caspase-3 mRNA by about 4 fold, as analyzed by RT-PCR at 24 hours (Fig. 4.1a). Increase in expression was evident after 12-hour doxorubicin treatment itself. In order to study the role of p53 in doxorubicin induced caspase-3 gene expression, a clone of MCF-7 cells expressing R273H mutant of p53 was used. These cells were treated with 500 ng/ml doxorubicin for 24 and 48 hours, RNA was isolated and RT-PCR analysis was done for caspase-3 and GAPDH. Caspase-3 mRNA levels did not increase in these cells upon treatment with doxorubicin (Fig. 4.1a). Instead, there was significant decrease in caspase-3 mRNA level upon doxorubicin treatment. Transcript levels of caspase-2, -6, -7, -8, and -9 did not change in the doxorubicin treated cells compared to the untreated control cells. Caspase-10 transcript levels were found reduced in doxorubicin treated cells (Fig. 4.1a).

In order to look at the effect of transient expression of p53 on caspase-3 gene expression, MCF-7 cells were transfected with pCB6 control plasmid or p53 expression plasmid. After 24 hours, RNA was isolated from these cells and analysed by RT-PCR for caspase-3 gene expression. p53 overexpression did not enhance the transcript levels of caspase-3 in MCF-7 cells (Fig. 4.1c). This result suggests that p53 alone is not sufficient to induce caspase-3 gene expression in these cells. Induction of caspase-3 gene expression by doxorubicin may require other signals in addition to p53 that are induced by doxorubicin.

### 4.2.2 Identification of p53-binding sites in caspase-3 gene

p53 dependent induction of caspase-3 gene by doxorubicin pointed to possibility of p53 binding to response elements in the caspase-3 gene, regulating its expression. Caspase-3 gene sequence was obtained from the draft human genome (Build 35.1) and gene structure was deduced by comparing it with caspase-3 mRNA sequence obtained from NCBI database (accession number NM_004346). The caspase-3 gene consists of 8 exons and spans about 22 kb in the q34 region of human chromosome 4 (Fig. 4.2a and Fig. 4.2b). The region upstream of first exon and first three introns were analysed for p53 binding sites using MatInspector software. The putative promoter region (upstream of 5'UTR) of caspase-3 gene identified using the PromoterInspector software did not have any p53 response element with high scores. However, in the third intron of caspase-3 gene, we identified two putative p53 binding sites (response elements). The site 1 has only two base mismatches (out of 20 bases) when compared to consensus p53 binding site while site 2 has three base pair mismatches. These putative p53 binding sites...
Figure 4.2. Identification of putative p53 response elements in caspase-3 gene.
(a) Caspase-3 gene region was identified using human genome blast at NCBI. Comparing the caspase-3 cDNA (accession number NM_004346) to the genomic region, the intron-exon structure of caspase-3 gene was identified as represented in (b). Analysis for putative p53 binding site was carried out using the Matinspector software that uses TRANSFAC database. The sequence of putative p53 binding sites and representative diagram of caspase-3 gene showing their position is given (a). * and # mark the translation initiation and termination sites respectively (c) The putative sites identified were cloned in the pCAT-promoter vector downstream of the reporter gene.
response elements from caspase-3 intronic region, along with a few flanking bases, were cloned in the BamHI site of the pCAT-promoter vector, downstream of the chloramphenicol acetyl transferase (CAT) gene, for reporter assays (Fig. 4.2c).

4.2.3 p53 mediated activation of reporter gene by intronic site 1

The reporter constructs with the response elements cloned were transfected into HeLa cells along with expression plasmids of p53 or R273H mutant of p53. Beta-galactosidase expression plasmid was also included as transfection control. After 30 hours, cell lysates were prepared using reporter lysis buffer and CAT assays were carried out. Co-expression of p53 along with site 1 reporter construct induced about 15 fold activation of CAT activity compared to control plasmid transfections while R273H mutant of p53 did not enhance the CAT activity (Fig. 4.3a). Similar results were obtained with site 1 that was cloned in opposite orientation in the pCAT-promoter vector (data not shown). Surprisingly, presence of site 2 did not enhance reporter activity when co-transfected along with p53 or mutant p53 (Fig. 4.3b). These results show that only site 1 is likely to be a functional p53-responsive site and putative site 2 is not a functional p53-responsive site.

4.2.4 Intronic sites 1 and 2 enhance p73 mediated reporter gene activity

As the putative p53 binding site 2 did not show any activation by p53, role of these response elements in mediating p73 dependent activation was examined. The reporter constructs with the sites cloned were transfected along with p73α, β, γ and δ expression plasmids and beta-galactosidase expression plasmid to HeLa cells. Cell lysates were prepared 30 hours after transfection and CAT assays were carried out. All the isoforms of p73 induced reporter gene activation in both the site 1 and site 2 constructs. Transfected along with site 1 reporter construct, p73 β and δ isoforms increased the CAT activity by about 75 and 89 folds respectively, whereas α and γ isoforms enhanced it only by about 8 and 13 folds respectively (Fig. 4.4a). Similar results were obtained using the reporter construct that had site 1 cloned in opposite orientation also (data not shown). The site 2 reporter construct showed about 3 and 6 fold increase in CAT activity with p73 α and γ respectively, while β and δ isoforms enhanced it by about 43 and 40 fold respectively (Fig. 4.4b). Empty reporter expression vector (without site 1 or site 2) did not show any significant increase in activity when co-transfected with p53 or p73 isoforms (Fig. 4.4c).
Figure 4.3. Effect of the putative site 1 and site 2 on p53 mediated reporter gene activity. (a) The reporter constructs with the response elements cloned were transfected along with pCB6 control plasmid or p53 or mutant p53 expression plasmids into HeLa cells. Beta galactosidase expression plasmids were included as transfection controls. 30 hours after transfection the cells were lysed and CAT activity and beta galactosidase activity were measured. The CAT activity measured was normalized using the corresponding beta galactosidase activity values and plotted. (b) Effect of p53 expression on reporter gene activity using the site 2 reporter plasmid was measured as in (a) and plotted. (C) Domain architecture of p53 and p73α, β, γ and δ isoforms. TA indicated transactivation domain, DB DNA binding domain and OD indicates oligomerization domain.
Figure 4.4. Effect of the putative site 1 and site 2 on p73 mediated reporter gene activity. (a) The site 1 and site 2 reporter plasmids, along with pCB6 control plasmid and expression plasmids for p73 isoforms were transfected into HeLa cells. Beta galactosidase expression plasmid was included as transfection controls. 30 hours after transfection the cells were lysed and CAT and beta galactosidase activities were measured. The CAT activities were normalized using the corresponding beta galactosidase activity values and plotted. (b) Effect of p73 expression on reporter gene activity using the site 2 reporter plasmid was measured as in (a) and plotted. (c) CAT activity in cells transfected with empty pCAT-promoter plasmid along with p53 and p73 isoform expression plasmids measured and plotted after normalization.
4.2.5 **Intronic site1 binds to p53 in vitro**

Since site1 enhanced expression of reporter gene in response to p53, its ability to bind p53 under in vitro conditions was tested. Oligonucleotides corresponding to the site were annealed and radiolabelled with $^{32}$P, incubated with nuclear extracts from doxorubicin treated MCF-7 cells and separated by PAGE. Binding of the oligonucleotides to p53 was observed as a shift in its migration after electrophoresis (Fig. 4.5a). This shift was competed out when binding reactions were carried out with 50-fold excess of unlabelled self and consensus oligonucleotides, but not with mutant oligonucleotides. Inclusion of a polyclonal p53 antibody into the binding reaction lead to abolition of shift due to p53 immunodepletion. These observations proved that site 1 interacts physically with p53.

4.2.6 **Doxorubicin does not induce caspase-3 gene expression in different cell lines**

Doxorubicin treatment was used to analyse caspase-3 gene expression under conditions of p53 activation in other cell lines. RNA was isolated from A549, HCT116, U2OS and HeLa cells treated with doxorubicin and RT-PCR was carried out to analyze the gene expression. Enhanced expression of caspase-3 gene was not observed in any of the tested cell lines in response to doxorubicin treatment (Fig. 4.6). A549, HCT116 and U2OS cell lines express wild type p53 and doxorubicin treatment of these cell lines has been shown to induce Ipaf gene expression (Sadasivam et al., 2005).

4.3 **Discussion**

We have identified induction of caspase-3 gene expression by doxorubicin in MCF-7 cells. This induction of caspase-3 gene expression was dependent on p53 as cells expressing mutant p53 did not show this upregulation. Analysis of caspase-3 gene using the human genome sequence revealed presence of two putative p53 response elements in the third intron of caspase-3 gene. Presence of p53 response elements in introns of target genes have been reported before. Genes for human death receptor 5 (Shetty et al., 2005), death receptor 4 (Liu et al., 2004), mismatch repair genes PMS2 and MLH1 (Chen and Sadowski, 2005), cyclin G (Zauberman et al., 1995), GADD45 (Carrier et al., 1996) and Puma (Nakano and Vousden, 2001) all have been reported to be activated by p53 through intronic response elements.

A p53 response element present in the intron of caspase-3 gene (site1) induced expression of CAT gene in response to p53 as well as p73 isoforms. In contrast to site1, p53 did not enhance the reporter activity when site 2 was incorporated into the reporter
Figure 4.5. Binding of p53 to the site 1 oligonucleotide. (a) The oligonucleotide corresponding to putative p53 binding site 1 of caspase-3 gene was radiolabelled with $^{32}$P and incubated with nuclear extracts of MCF-7 cells treated with doxorubicin. Lane 1 is the binding reaction without nuclear extract and lanes 2-7 are with nuclear extracts. A shift was observed in the mobility of the oligonucleotide (lane2) which was abrogated upon incubating the lysate with unlabelled site 1 oligonucleotide (lane3) or with oligonucleotide corresponding to consensus p53 binding site (lane 5), but not with mutated site oligonucleotide (lane 4). Incubation of lysates with a polyclonal anti-p53 antibody (lane 6), but not PBS (lane 7), led to immunodepletion mediated abolition of the shift. The sequence of site1, its mutant and consensus p53 binding sites used are given in (b)
Figure 4.6. Effect of Doxorubicin on caspase-3 gene expression in U2OS, A549, HeLa and HCT116 cells. The cells were treated with doxorubicin for indicated periods of time and RNA was isolated from them. RT-PCR analysis was carried out to determine levels of caspase-3 and GAPDH transcripts.
vector. However site 2 was responsive to p73, although the increase in activity was less compared to site 1. Exact reason for difference in the activity of the two response elements with respect to p53-mediated transcription is not known. Site 2 differs from the consensus p53 binding site at three positions compared to two mismatches in the case of site 1. Since core sequences in site 1 and 2 are identical, the difference is likely to be due to purines/pyrimidines flanking the core sequence.

Caspase-3 gene expression in response to doxorubicin was analysed in other cell lines in order to look at the general nature of p53-mediated regulation of caspase-3 gene expression. None of the tested cell lines showed increased caspase-3 mRNA levels in response to doxorubicin treatment. This may point to cell-specific regulation of caspase-3 gene expression by p53 in MCF-7 cells. However, a recent study has reported that caspase-3 gene, along with several other caspase genes, is upregulated in HT1080 cells in response to doxorubicin treatment (Lehnhardt et al., 2005). As RT-PCR detects steady state level of mRNAs in cells, there is a possibility that in many cells, there might exist enhanced transcription coupled with high levels of mRNA degradation under apoptotic conditions induced by doxorubicin. Run on assays that measure levels of transcription might help to clarify this point.

Over expression of p53 in MCF-7 cells did not induce expression of caspase-3 gene. This points to requirement of another factor for p53-induced transactivation of the gene. Ipaf, an activator of caspase-1, also shows such a requirement for p53-mediated expression in MCF-7 cells (but not in A549 cells) whereas caspase-1 does not. This points to possible induction of a p53 co-activator in MCF-7 cells upon doxorubicin treatment that is required for upregulation of certain genes. Overexpression of PTP-S2 in MCF-7 cells does not induce caspase-3 gene expression, possibly due to difference in the regulation of p53 mediated transcription in response to diverse stimuli.

It is of interest to note that p53 upregulates caspase-6 expression in several cell lines, but not in MCF-7 cells (MacLachlan and El-Deiry, 2002). Similarly, even though caspase-8 promoter and caspase-10 gene expression is regulated by p53, we did not observe increased expression of this gene by doxorubicin in MCF-7 cells (Liedtke et al. 2003, Rikhof et al., 2003). However, caspase-1 transcription is regulated by p53 in these cells (Gupta et al., 2001). This might point to requirement of additional factors or p53 modifications that are required for this function that is not present in all cell types. Tissue specific regulation of apoptotic target genes by p53 has already been reported in murine system (Fei et al. 2002).
Increased expression of caspase genes is known to facilitate and accelerate apoptosis (MacLachlan and El-Deiry, 2002; Monroe et al., 2002; Pompl et al., 2003). Caspase-6, which is a p53 target gene, has been shown to reduce the threshold for p53 dependent apoptosis. Thus it is likely that upregulation of caspase-3 gene by p53 may sensitize cells to p53-induced apoptosis. Effect of p53 mediated caspase-3 gene regulation on apoptosis could not be studied as caspase-3 gene has a 47 bp deletion in the third exon in MCF-7 cells, leading to abrogation of its translation (Janicke et al., 1998a).

### 4.3.1 Upregulation of caspase-3 gene expression by apoptotic stimuli

Although caspase-3 is almost ubiquitously expressed, several apoptotic stimuli have been shown to enhance expression levels of caspase-3 gene. X-ray irradiation of human squamous cell carcinoma cell line SAS induced caspase-3 gene expression in p53-dependent manner (Yasumoto et al. 2003). Caspase-3 expression is upregulated under conditions of neuronal hypoxia in several systems (Chen et al. 2000; Schmidt-Kastner et al. 2000; Harrison et al. 2001). Cytotoxic drugs also have been shown to upregulate caspase-3 gene expression in different cells (Droin et al., 1998, Lehnhardt et al., 2005). Caspase-3 mRNA upregulation in tail muscle and spinal cord of *Xenopus tropicalis* during tail regression has been correlated with appearance of TUNEL positive cells, indicating a role in apoptosis (Rowe et al. 2002). Rat caspase-3 promoter has been cloned and characterized (Liu et al. 2002). Analysis of this promoter revealed role of Sp1 in regulation of caspase-3 gene expression. A putative ETS-1 site was also described as important in regulation of the promoter activity. No putative p53 or p73 responsive site has been reported in rat caspase-3 promoter.

### 4.3.2 Caspase-3: role in apoptosis

Caspase-3 is the best studied executioner caspase and plays a major role in apoptosis by cleaving several proteins. Important targets of caspase-1 include fodrin, gelsolin, U1 small nuclear ribonucleoprotein, DNA fragmentation factor 45 (DFF45)/inhibitor of caspase-activated DNase (ICAD), receptor-interacting protein (RIP), X-linked inhibitor of apoptosis protein (X-IAP) and topoisomerase I among other proteins (Slee et al., 2001). However, caspase-3 and caspase-7 have been shown to be functionally redundant, at least to some extent; they have similar substrate specificity and can proteolytically process similar set of proteins (Thornberry et al., 1997; Janicke et al., 1998b). Expression levels and function of caspase-7 has been shown to dictate viability of caspase-3-/- mice (Houde et al., 2004).
Although caspase-3 plays a major role in execution phase of apoptosis, its activity is not absolutely essential for apoptosis in cells. MCF-7 that lack caspase-3 protein undergoes apoptosis in response to various stimuli, but does not exhibit DNA fragmentation and some morphological features of apoptosis in response to several stimuli (Janicke et al., 1998a). However, reconstitution of caspase-3 in MCF-7 cells led to enhanced apoptotic response to doxorubicin, etoposide as well as ionizing radiation (Yang et al., 2001, Essmann et al., 2004; Yang et al., 2005).

In addition to its well-studied role as an executioner caspase, caspase-3 has been attributed certain other apoptotic functions. Caspase-3 activity is necessary for cytochrome c release and caspase-9 activation in response to various apoptotic stimuli in MCF-7 cells (Blanc et al., 2000; Radha et al., 2002; Essmann et al. 2004). Ability of caspase-10 to sensitize MCF-7 cells to TRAIL induced apoptosis depends on the presence of functional caspase-3 (Engels et al. 2005). During granzyme B mediated cell death, caspase-3 activity is necessary for mitochondrial depolarization and functions downstream of Bax/Bak function (Metkar et al., 2003). Caspase-3 plays a major role in the feedback loop that induces mitochondrial damage, leading to enhanced response to cell death (Ricci et al., 2003; Ricci et al., 2004). Thus caspase-3 is an important protein that functions to amplify the apoptosis signals in cells.

In this study we have identified a role for p53 in caspase-3 gene expression in MCF-7 cells induced by doxorubicin. We have also identified a putative regulatory element in the intron of caspase-3 gene that may be responsible for p53-mediated regulation. However, caspase-3 function is absent in MCF-7 cells due to a deletion in its gene and effect of this regulation on the apoptosis could not be studied. Caspase-3 expression is downregulated in a number of breast cancers (Devarajan et al., 2002). As caspase-3 function plays a major role in regulating sensitivity of MCF-7 cells to a variety of apoptotic stimuli as well as in the activation of caspase-9, regulation of its expression might function in priming cells to apoptosis.