4.1. Introduction

Survivin is one of the most cancer-specific proteins identified to date, being upregulated in almost all human tumours. Biologically, survivin has been reported to inhibit apoptosis, enhance proliferation and promote angiogenesis. Because of its upregulation in malignancy and its key role in apoptosis, proliferation and angiogenesis, survivin is currently attracting considerable attention as a new target for anti-cancer therapies. In several animal model systems, down regulation of survivin or inactivation of its function has been shown to inhibit the tumour growth (Ryan et al., 2009; Li, 2003; Altieri, 2008). Survivin is an acidic 16.5 KDa protein with 142 amino acid residues and is composed of a single *Baculovirus* inhibitor of apoptosis repeat (BIR), an evolutionary conserved motif domain at the N-terminal region and an extended C-terminal α-helix turn helix domain. Further the occurrence of ring finger domain is a unique feature of other inhibitor of apoptosis proteins (IAPs) also reported in survivin (Ambrosini et al., 1997). Survivin was originally described as an anti-apoptotic protein that is overexpressed in a variety of human cancers and hence designated as a cancer-specific protein. This protein is expressed at a high level during fetal development and rarely expressed in normal healthy adult tissues; however, upregulated in majority of cancers (Wimmershioff et al., 2010).

Wheatly *et al* significantly pointed out that the survivin is a bifunctional protein that suppresses apoptosis and regulates cell division for cancer biomarker (Wheatly *et al.*, 2005). Consistent with its role in inhibition of apoptosis and promotion of cell proliferation, survivin plays a key role in cancer progression (Brid *et al.*, 2009; Altieri, 2003). Because of its potential role in cancer, an intensive investigation is going on in elucidation of structure and functional features of survivin to address it’s potentially as a suitable tumour “Biomarker” (Duffy *et al.*, 2005).
2007). In addition the survivin blocks apoptosis is clearly demonstrated by inhibiting active caspase activity (Li et al., 2006).

Survivin is reported to regulate the cell cycle, particularly at G2/M phase in dividing cells. Cytologically survivin is incorporated into centrosome and mitotic spindles and finally relocated to midbodies in the late telophase (Susanne et al., 2006). Survivin-deficient cells initiate cell division process but failed to complete cytokinesis; apparently because the spindle midzone and midbody microtubules are absent during late mitosis (Dun et al., 2004). Gassmann et al., (2004) noticed the survivin in the mitotic apparatus, centrosomes, kinetochores, mitotic spindle fibers, spindle poles, central spindle midzone and midbodies and other known subcellular localizations are cytosol, mitochondria and nuclei (Colnaghi et al., 2006). In several studies, it is clearly emphasized that the survivin has a regulatory function during cell division. For instance, Yeast cells with knockout of certain IAP genes did not show any symptoms for cell death, however, it showed defects in mitosis characterized by improper chromosome segregation or failed cytokinesis (Chan, 2000).

**Scheme:** Schematic representation of survivin protein structure. BIR: Baculovirus IAP repeat; INCENP: Inner centromere protein; NES: Nuclear export signal; PKA: Protein kinase A; XIAP: X-linked inhibitor of apoptosis protein.

The chromosomal passenger complex (CPC) is a key regulator of Chromosome segregation and cytokinesis are based CPC functions connected to its localization. The complex
first localized in centromeres and later associated with the central spindle fiber and midbody (Earnshaw, 2005). Survivin, Borealin and INCENP (inner centromere nuclear protein) are the three components of the CPC that regulate the activity and localization of its enzymatic component, the kinase Aurora B (Resnick et al., 2006). Survivin appears to be a component of the chromosomal passenger protein complex that participates in multiple facets of cell division (Whealey et al., 2001). Survivin interacts with Aurora B and inner centromere protein (INCEP) (Reiko et al., 2003). This complex Aurora B/INCEP/Survivin binds centromere of metaphase chromosome at the central spindle midzone at the anaphase chromosome, which is a characteristic of chromosomal passenger proteins that actively participate in chromosome segregation and cytokinesis (Kaitna et al., 2000; Sha et al., 2006). In addition to survivin, there are several other intracellular proteins that are known to restrain cell death when highly expressed, such as, for example, Bcl-2, Bcl-xL, c-IAP2, XIAP, and FLIP, which also have been found overexpressed in many tumours. Considering the well-known function of survivin as an inhibitor of caspases and, consequently, as an anti-apoptotic protein, it is not surprising that down-regulation of this protein by associated with increased cell death. It has been shown in several other experimental systems that the down-regulation of survivin expression, for example by nano-biotechnological approaches.

Most anticancer treatments are believed to induce tumour regression, at least in part, by mediating apoptosis (Fesik, 2005; Pennati et al., 2007). Indeed, an inability to induce apoptosis may be partially responsible for resistance to several anti-cancer therapies ((O’Connor et al., 2000; Conway et al., 2003). Consistent with this hypothesis the present study has been designed to assess the over expression of survivin in selected tumour cells and determination of the failure of cytokinesis, generation of multinucleated and apoptotic cells.
The main objectives of this chapter are as follows:

- To find out the survivin expression in different selected human cancer cell lines (MCF-7, A549, ZR751) and Normal HBL100 cells.
- To target and inhibit the survivin up-regulation in selected human breast cancer MCF-7 cell by anti-cancer drug Oxaliplatin.
- To determine the apoptotic effect of Oxaliplatin treated cancer cells.
- To evaluate the impact of chemotherapeutic drugs like Oxaliplatin to target survivin expression.

### 4.2. Materials and Methods

#### Materials

MTT-3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-terazolium bromide, Acridine orange, ethidium bromide, DAPI (4′, 6-diamidino-2-phenylindole, dihydrochloride) stain, and dimethyl sulfoxide (DMSO) purchased from Himedia Chemical Pvt. Ltd. DMEM medium, fetal bovine serum and penicillin/streptomycin were purchased from (Sigma). All organic solvents and other chemicals were of analytical grade or complied with the standards needed for cell culture experiments were purchased from Himedia, Chemical (India).

#### Cell Cultures and reagents

The cancer cell lines namely Human breast adenocarcinoma cell line (MCF-7), ductal breast carcinoma cell line (ZR-75-1), Human lung carcinoma cell line (A549) and Normal human breast cell (HBL100) line were procured from National center for cell science (NCCS, Pune) and revived in 20% DMEM medium containing 10% foetal bovine serum (FBS). The culture medium also reconstituted with 2 mM L-glutamine, 1 mM Sodium Pyruvate and 1mM pencillin and streptomycin. The cultures were maintained in t-25mm flask with the growth condition maintained at 37°C and 5 % CO2 in an air jacketed CO2 incubator. After the cells
attained 70-80% confluency trypsinized and seeded in 96mm Petri plates of tissue culture grade for further experimentation and the media changed 24h of cells. Further, the medium contamination was avoided by subculturing the cells at an appropriate time. The DMEM containing 10% FBS need only to be used after sub-culturing. When the cells attained above 80% confluency, the culture medium was gently removed. Cells were then washed twice with PBS EDTA (pH 7.4). Fresh medium containing 10% FBS was freshly added and resuspended gently using a Pasteur pipette. Cells were counted using haemocytometer. A fixed number of cells were seeded into Petri plates and maintained until used for further studies.

**Cell Fractionation, SDS-PAGE and Western Blotting**

Whole-cell extracts were prepared by scraping cells off Petri dishes, washing cell pellets twice in PBS, and then resuspending cell pellets in two-packed cell volumes of RIPA buffer [150 mM NaCl/ 50mM Tris-HCl, pH 7.5/0.25% (wt/vol) deoxycholate/1% Nonidet P-40/ 5mM sodium orthovanadate-2mM sodium fluoride-protease inhibitor mixture]. Nucleus and cytoplasmic components were isolated separately from each cell lines after washing cell pellets in buffer A (10 mM Hepes, pH 7.5/ 10mM KCl/ 1.5mM Mg2Cl/ 0.5mM NaF-1mM glycerol phosphate-protease mixture), then lysis in buffer A / B (buffer A plus 0.5% Nonidet P-40) in a 2:1 ratio. After centrifugation (12,000 rpm for 10 min), supernatant was collected (cytoplasmic fraction). The Pellets were washed again in PBS centrifuged (12,000 rpm for 10 min), and then flash-frozen in dry-ice–ethanol in buffer C (20 mM Hepes, pH, 7.5/ 420mM NaCl/ 1.5mM Mg2Cl/ 0.5mM NaF/ 0.5mM DTT/ 1mM glycerol phosphate-protease mixture), followed by a slow thaw on ice. The supernatant was centrifuged (12,000 rpm) and collected (fraction).

Protein concentrations were determined by following a modified Bradford method (Bio-Rad). Equal amounts of proteins (50 µg) were resolved by 12% SDS-PAGE under reducing
conditions. Proteins were then transferred to nitrocellulose membranes. Efficiency and equal loading of proteins were evaluated by Ponceau S staining. Membranes were blocked for 1 h in TBS (25mM Tris/HCl, pH 7.4/ 150mM NaCl/ 2.7mM KCl) containing 4% (wt/vol) low-fat milk or 3% BSA (wt/vol). Membranes were then probed with specific Anti-human survivin antibody recognizing target proteins and proteins were visualized with the Super Signal West Femto maximum sensitivity substrate kit (Pierce).

**RNA isolation and cDNA synthesis**

Following treatment, cells grown in 60 mm Petri dishes were washed with ice-cold PBS and 1 ml of trizol was added and flushed gently to disrupt the cells. The lysates were collected and mixed with 300µl of chloroform by inversion. The tubes were then centrifuged at 10,000 rpm for 15 min at 4°C. The aqueous phases from the tubes were collected and the RNA was precipitated using 700µl of isopropanol and centrifuged at 10,000 rpm for 10 min at 4 °C. The pellets were washed twice with 70% ethanol and air-dried for about 20-40 min. The pellets were resuspended in 100µl of DEPC treated water. The purity of RNA was checked by OD260/280 of RNA samples (Nanodrop, Thermo Inc). The quality of the RNA was analyzed by agarose gel electrophoresis. Isolated total RNA (1µg) was reverse-transcribed to cDNA in a reaction mixture containing 4µl of 5x reaction buffer, 2 µl of dNTPs mixture (10 mM), 20 units of RNase inhibitor, 200 units of avian-myeloblastosis virus (AMV) reverse transcriptase and 0.5µg of oligo (dT) primer in a total volume of 20µl. The reaction mixture was incubated at 42°C for 60 min and the reaction was terminated by heating at 70°C for 10 min. The resultant cDNA was stored at -80°C until further use.
PCR amplification

All oligonucleotide primers were purchased from invitrogen Bioservices, India. Details about the primers are given in Table. The PCR amplification reaction mixture (in a final volume of 25 µl) contained 1µl of cDNA, 0.5 µl of forward primer, 0.5 µl of reverse primer and 10µl of Hot Master Mix (2.5x). The PCR was carried out in a thermal cycler (Eppendorf). Negative controls without cDNA were also performed. Amplification products were analyzed by electrophoresis in a 2% agarose gel containing ethidium bromide with 100 bp DNA ladder. The PCR products were visualized as bands with a UV-transilluminator and photographs were taken using gel documentation system (GelDocMega™, United Kingdom)

The expression of survivin mRNA was analyzed by semi-quantitative RT-PCR. The correlation between band intensity and dose of cDNA templates was linear under the conditions described below. Total RNA was extracted from the selected cancer as well as normal cells using TRlzol reagent (Invitrogen), respectively and RNA of 2 µl (1 µg/ µl) was used to synthesize cDNA using Superscript First-Strand Synthesis Kit (Promega) following the manufacturer’s protocols. The cDNA was further used to amplify the survivin mRNA fragment, while the housekeeping gene β-actin was also amplified as an internal standard. The corresponding primer sequences were depicted in Table: 1.1 as follows:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivin</td>
<td>Forward: 5’-TTCTCAAGGACCACCGCAGTC-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’-AGAGGCCTCAATCCATGG-3’.</td>
</tr>
<tr>
<td>β-actin</td>
<td>Forward: 5’-AGCAACCGGAGCTGTTG-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5’ CATTCCGAAGT-G-3’.</td>
</tr>
</tbody>
</table>

The PCR cycling program was performed as follows: 1 cycle of 94ºC for 3 min; 35 cycles of 94ºC for 40 s, 62ºC for 40 s, and 72ºC for 90 s; followed by a final elongation step of 72ºC for 10 min. Then RT-PCR products were electrophoresed through a 1.5% agarose gel with
ethidium bromide. Signals were quantified by densitometry analysis using the Lab works Image Acquisition (UVP, Inc., Upland, CA).

**In Vitro Cytotoxicity Assay**

The culture flask cells were then subjected for MTT assay. The stock concentration (5mg/mL) of MTT-(3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a yellow tetrazole) was prepared and 100µL of MTT was added in each Oxaliplatin treated wells and incubated for 4h. Purple color formazone crystals were observed and these crystals were dissolved with 100µL of dimethyl sulphoxide (DMSO), and read at 620nm in a multi well ELISA plate reader (Thermo, Multiskan). OD value was subjected to sort out percentage of viability by using the following formula,

\[
\text{Percentage of viability} = \left( \frac{\text{Mean OD value of experimental sample (Oxp)}}{\text{Mean OD value of experimental control (untreated)}} \right) \times 100
\]

**Morphological Observation**

ZR751, A549 and MCF-7 cells were grown (1×10^5 cells/cover slip) and incubated with Oxaliplatin at their IC\textsubscript{50} concentration and then they were fixed in methanol: acetic acid (3:1, v/v). The cover slips were gently mounted on glass slides for the morphometric analysis. Morphological changes of cells were analyzed under the Nikon (Japan) bright field inverted light microscopy at 40x magnification.

**Acridine orange (AO) and Ethidium bromide (EtBr) staining**

Approximately 5µL of dye mixture [(100 mg/mL Acridine Orange (AO) and 100 mg/mL Ethidium Bromide (EtBr)] was mixed with 9mL of cell suspension (1x10^5 cells/mL) on a clean microscopic cover slip. After incubation for 2-3 min, cells were visualized under fluorescence microscope (Nikon Eclipse, Inc, Japan) at 40x magnification with excitation filter at 510-590 nm.
**DAPI (4′, 6-diamidino-2-phenylindole, dihydrochloride) staining**

Cells were treated with Oxaliplatin for 48 hours, and then fixed with methanol: acetic acid (3:1, v/v) prior to washing with PBS. The washed cells were then stained with 1 mg/mL DAPI (4′, n6-diamidino-2-phenylindole, dihydrochloride) for 20 minutes in the dark. Stained images were recorded with fluorescent microscope with appropriate excitation filter.

\[
\text{Percentage of apoptotic cells} = \frac{\text{Total number of apoptotic cells}}{\text{Total number of normal and apoptotic cells}} \times 100
\]

**Haematoxylin and Eosin staining method**

Haematoxylin and Eosin cell staining was conducted for qualitative evaluation of cellular morphology and to determine the mitotic indices for quantitation of cell cycle shift and abnormal morphology. Cells were seeded on heat-sterilized cover slips in 6-well plates at a density of 250,000 cells per well and allowed to attach overnight. Cells were subsequently exposed to Oxaliplatin and appropriate controls respectively for 48h, after which cover slips were fixed with Bouin’s fixative (30 min) and 70% ethanol (20 min). Cover slips were rinsed with tap water, stained with hematoxylin (20 min), rinsed (tap water and 70% ethanol) and stained with eosin (7min). Cover slips were then dehydrated stepwise with ethanol (70%, 96%, 100%) and xylene, mounted on microscope slides with Entellan resin and dried overnight. Qualitative evaluation (100× and 400× magnification) was conducted with a Zeiss inverted Axiovert CFL40 microscope and photomicrographs were taken with a Zeiss Axiovert MRm monochrome camera (Carl Zeiss MicroImaging, Inc., NY, USA). Mitotic index counts were determined, with individual cells being divided into different phases of the cell cycle based on morphological characteristics. Cells which could not be categorized due to excessive fragmentation, highly unusual nuclear morphology or lack of clear nuclear material were defined and counted as ‘abnormal’. The experiment was repeated three times and 1000 cells were counted per slide.
Data was converted to represent percentages of cells present in each defined category providing representative statistics.

*Multinucleation index measurements*

For the assessment of the multinucleation/mitotic indexes, a total of 1,000 cells were scored for each time point. The multinucleation index was calculated by dividing the number of multinucleated interphase cells by the total number of interphase cells.

*Statistical analysis*

All measurements were made in triplicate and all values were expressed as the mean ± standard error of the mean. The results were subjected to an analysis by Student's t-test. The results were considered statistically significant if the p value was ≤0.05.

4.3. Results

Survivin expression was found to be absent in normal and non-malignant cells. Whereas survivin was overexpressed in all the cancer cells and contributed towards phenotypic variation in cancer cells. 12% SDS-PAGE analysis clearly showed that the cellular extract of the selected cancer cell lines MCF-7 Breast cancer, ZR-75-1 ductal breast carcinoma, A549-Lung carcinoma and HBL100-Normal breast cells contained a 16.5 kDa protein. As shown in (Figure 1.1) protein band was observed to be very low in control HBL100 normal human breast cells. Thus the results depicted in Figure 1.1 authentically proved that the survivin was found to be overexpressed in cancer cells when compared with control. Among the three selected cancer cell lines survivin protein was comparatively higher in MCF-7 followed by A549 and ZR-75-1 cells. This finding attributes much more to target survivin as a biomarker for breast cancer.

The result shown in Figure 1.2 experimentally confirmed the over expression of survivin protein by adopting western blot analysis. In addition the western blot staining of survivin with
its monoclonal antibody raised in rabbit well documented the appearance of additional band just beneath the survivin band. This proteomic data may suggest that the survivin was found to be synthesized in cancer cells throughout their life span. The band noticed at the top must be the fully matured protein released into the cytosol after post translational modification process. Meanwhile, the band located just below the survivin may be the immature peptide of survivin which was extracted from cells either before or at the time of post translational modification. Thus, the Figure 1.2 proved that the cancer cells led to the improper synthesis and maturation of proteins particularly the survivin. It was very surprised to see that the internal standard β-actin was expressed in almost equal quantity in all the cells irrespective of their category and types of cancer. This observation obviously bring a novel idea to designed and target the survivin as a suitable cancer biomarker. It was also noticed that the expression of survivin in ZR-75-1 cells was poor when compared to the rest of the two cancer cells namely MCF-7 and A549. Furthermore, the immature band was not detected in ZR-75-1 and similar trend was noticed in the control breast cells (HBL 100).

The selected cell lines namely HBL-100, ZR-75-1, A549 and MCF-7 cells were subjected to extraction and isolation of survivin mRNA consistent with this isolated mRNA was subjected to agarose gel electrophoresis at the concentration of 1.9%. The result depicted in Figure 1.3 significantly proved that considerable amount of mRNA was found to be higher in MCF-7 cells and the mRNA content was gradually decreased in the following order such as A549, ZR-75-1 and HBL100 respectively. The observation made in cell lines showed that there is no significant discrimination between the control and the cancer cells the cytotoxic variation was also noticed in all the selected cancer cell lines. The level of variation is an index for the level of expression of survivin in the respective cells. Hence, the present observation clearly authenticate that the
RNA concentration variation is the common factor to determine the level of expression of survivin.

The isolated mRNA was used as templates for the synthesis of survivin cDNA using RT-PCR. The data shown in Figure 1.4 revealed agarose gel electrophorogram with a distinguishable bands for survivin cDNA. The RT-PCR amplicon for survivin was found to be more prominent in MCF-7, A549 and ZR-75-1 cancer cells and a weak band was noticed for the normal breast cell (HBL100). Thus, the above mentioned evidences clearly confirmed the expression of survivin in all the selected cell lines irrespective of their origin. Furthermore, the band intensity showed discrimination between the cancer and non cancer cells.

MTT assay was performed to determine the cytotoxic effect of Oxaliplatin on ZR751, A549 and MCF-7 cells. Fifty percentage of cell death, which determines the inhibitory concentration (IC$_{50}$) value of Oxaliplatin against ZR-75-1, A549 and MCF-7 cells holds at 5µM/mL, 6µM/mL and 4µM/mL (Figure 1.5). Thus the present findings proved that the Oxaliplatin found to have more toxic effect at above IC$_{50}$ value in the normal cells also. A large number of in vitro studies indicated that the oxaliplatin are toxic to the mammalian cells. Significantly, the conclusive evidence for cytotoxic effect of Oxaliplatin against the selected cancer cell lines. The morphological changes of ZR-75-1, A549 and MCF-7 cells treated with Oxaliplatin for 24hr were analyzed by light microscope which showed an important apoptotic changes such as membrane blebbing, appearance of monolayer, shrinkage of plasma membrane were observed. Occasionally clumped cells debris’s may also be noticed in all the selected cell lines treated with Oxaliplatin. Light and Fluorescence microscopy results of IC$_{50}$ concentration of Oxaliplatin treated A-549 cells shown in Figure 1.6. The cytotoxicity was observed under light microscope (a and b) which showed the disintegration of cells and aggregation of dead
cells. Further, the cells morphology was significantly changed like sickle shape or irregular cell margins. Fluorescence microscopy study of AO/EtBr staining of respective cells appear in live cells in green colour and orange colored apoptotic cells and necrotic cells appearing in red colour as indicated by arrows (c and d). The DAPI nuclear staining of treated cells exhibited condensed form of nuclear materials in apoptotic cells (e and f). Light and Fluorescence microscopy images of IC50 concentration of Oxaliplatin treated ZR-75-1 and MCF-7 cells shown in Figure 1.7 and Figure 1.8.

Furthermore, the Oxaliplatin treated cancer cells exhibited emblematic changes of apoptosis such as cell shrinkage, nuclear condensation and fragmentation. Interestingly, the apoptotic nuclei was found to have highly condensed chromatin which was observed blue fluorescent colour after staining with DAPI (4’, 6-diamidino-2-phenylindole) stain. The stained cells seems to be bright blue in falcate nucleus, the entire chromatin was present in one or a group of featureless, globular beads. In the case of drug treated cells the nucleus was noticed bright scattered amorphous structure. The total number of apoptotic cells was calculated from DAPI staining (Figure 1.9). The significant apoptotic cells present in the MCF-7 cancer cells showed Oxaliplatin was more effective in breast cancer cells. The present study investigated that the selected cancer cells leads to the multi nucleated conditions with condensed DNA. Cells showed two nuclei or one bilobed nucleus (multinucleated conditions) including mitotic arrest and there was no telophase, lead to failure of cytokinesis. To distinguish among these possibilities, we further examined the nuclear morphology of the survivin down-regulated cells by Haematoxylin and Eosin staining.

The light microscopic observation of normal and cancer cells scientifically proved that the normal breast cell line namely HBL100 found to have mononucleated cells and few cells
were found to be binucleated. Whereas, in cancer cells mostly observed binucleated, trinucleated and multinucleated cells were noticed. The A549 lung adeno carcinoma cells found to contain 3 to 6 nucleated cells. By contrast the MCF-7 cells were found to contain 2 to 10 nuclei in a single cell. The appearance of multinucleated condition is a phenomenon of improper segregation/imparalized cell division process taking place in survivin deficient cancer cells (Figure 1.10). The histological study experimentally proved the concept that the deficiency of survivin is indirectly proportional to the number of nuclei appeared in cancer cells that is the number of nuclei were found to be increased with decreasing the concentration of survivin. The total number of multinucleated cells was counted and higher significant counts of multinucleated cells were observed in MCF-7 cells (Figure 1.11).

The abnormal number, size and morphology of nuclei indicated that the survivin depletion causes defects in mitosis and/or cytokinesis. The presence of multinuclei in survivin depleted cells suggested that segregation of mitotic chromosomes has been disturbed. The defect in cytokinesis that arises in the absence of survivin was examined in survivin depleted cells which was confirmed by western blot (Figure 1.12). The most dramatic phenotype observer in mitotic down-regulated survivin expressed cells was a highly penetrate failure of cytokinesis, as observed by scoring the percentage of multinucleate cells. Hence, this present study tentatively concludes that the involvement of survivin during cell division makes the protein essential for completion of cytokinesis in all the selected mammalian cancer cell lines.
**Schmatic I**: The diagramatic representation of the mode of action of Oxaliplatin at cellular level. This diagram celarly shows that the Oxaliplatin drug specifically target the genomic DNA and induce apoptotic event.

### 4.4. Discussion

The inhibitor of apoptosis proteins (IAPs), which are able to inhibit apoptosis induced by a variety of stimuli, are always overexpressed in cancer cells that give rise to resistance to apoptosis. IAPs are a class of structurally related proteins that were initially identified in baculoviruses. At present, eight human IAPs have been identified: c-IAP-1, c-IAP-2, ILP-2, neuronal apoptosis inhibitory protein (NAIP), X-linked IAP (XIAP), Survivin, Livin, and Apollon/Bruce. Survivin is functioning as a chromosomal passenger protein, ensuring the proper alignment of chromosomes during mitosis and allowing for equal and complete cell division (Vagnarelli et al., 2004). IAPs are defined by one or more repeats of a highly conserved ~80 amino-acid zinc-binding domain located at the amino-terminus, termed the baculovirus IAP repeat domain. It has been well proved that the IAPs block apoptosis either by binding and inhibiting specific caspases, or through caspase-independent mechanisms. In addition to that the IAPs exhibit other biological functions that include involvement in protein degradation, and c-
IAP1 and c-IAP2 are integral parts of the type-2 TNF-receptor complex, while Survivin plays a role in cell mitosis, mainly in microtubule organization.

Survivin involves in at least three homeostatic networks: the control of mitosis (1), the regulation of apoptosis (2), and the cellular stress response (3). This classification is not restrictive, as novel functions of survivin are continuously proposed, as well as new roles for known properties. Even within the same network, survivin performs multiple roles. For instance, at mitosis, survivin acts as a passenger protein (Ruchaud et al., 2007) for proper chromosomal alignment, controls chromatin-associated spindle formation (Sampath et al., 2004), enhances spindle stability via suppression of microtubule dynamics (Rosa et al., 2006), and oversees kinetochore-microtubule attachment in the spindle assembly checkpoint (Sandall et al., 2006).

Cell division involves coordinated chromosomal and cytoskeleton rearrangements to ensure the faithful segregation of genetic material into the daughter cells (Endoh et al., 2001). The chromosomal passenger complex (CPC), survivin has emerged as a central player at several steps in this process (Angell et al., 2008). Studies have shown that survivin interacts with both aurora-B and INCENP forming a CPP complex though to be essential to cytokinesis which is imparalised according to our findings described here. To investigate this possibility, we examined the expression of survivin in normal and selected cancer cells (Figure. 1 and 2). The down-regulation of survivin expression in cancer cells to displayed abnormalities in chromosome segregation including lagging chromosomes and DNA bridges (Uren, 2000; Castedo et al., 2004). The DNA bridges failed to resolve during telophase and this failure was presumably a means by which cells with multinuclei produced. This report was in consistent with our finding which is shown in (Figure. 8) the multinuclear condition is an index of expression of survivin. Depletion of survivin leads to chromosomal nuclear multiplication and lack of cytokinesis.
Furthermore our results pointed out that the lagging chromosomes represent sister chromatid that have been left behind, rather than the product of sister chromatid non-disjunction. There is compelling evidence that defects in apoptosis contribute to human diseases. In the case of cancer, an abnormally increased cellular lifespan as a consequence of reduced apoptosis is ideal to favor the insurgence of genetic mutations, as well as to shield transformed cells from death induced by chemotherapy or radiotherapy, and to promote their survival at distant sites. Therefore, it is not surprising that manipulation of apoptosis has emerged as a new therapeutic strategy to preserve tissue integrity or to help eliminate cancer cells. This present study focus on survivin, a recently described molecule that appears to function as an integrator of both cell death and mitosis, and its potential impact in cancer diagnosis and therapy.

Survivin is a well known regulator of a cell cycle. Survivin enhance the levels of cell cycle inhibitors in cancer cells (Wang et al., 2004). Interestingly, the western blot date gains support from earlier findings of Koppler et al., 2005. They stated level of p53 was suppressed in the presence of survivin. In conclusion, attribute the defects in mitosis and cytokinesis to disruption of the survivin CPP complex (Vader et al., 2006). It may be Mitochondria-targeted survivin was involved in the formation of tumours, but cytosolic survivin inhibits tumour growth (Liu et al., 2004). The present investigation concluded that the use of different cancer cell lines that vary in their multinucleated conditions when compare with normal cell line. It seems reasonable to suspect that abnormal expression or function of survivin might contribute to apoptosis (Vong et al., 2005). Besides the outcome of the present study may provide a possible reason to elucidate the mechanism of action of survivin in cells. Since survivin was regulating the cell cycle and essential protein for the completion of cytokinesis. Results from our study showed down-regulation of survivin and also inhibit cytokinesis (Figure. 10). Earlier evidence
report that depletion of survivin in cancer cells causes of decrease cytokinesis activity and increased karyokinesis further supports our speculation.

There is evidence that survivin plays an important role in the drug-resistant phenotype of human cancer cells. Giodini et al, first reported that infection of HeLa cells with an adenoviral vector expressing survivin suppressed apoptosis induced by taxol (Giodini et al., 2002). Subsequently, we found that stable transfection of human ovarian cancer cell lines with survivin cDNA was able to protect them from the cytotoxic effects induced by taxol and taxotere (Zaffaroni et al., 2002). Zhang et al showed that forced expression of wild type survivin in human prostate cancer cell lines increased resistance to taxol in vitro and in vivo. In the clinical setting, when we analyzed the response of advanced ovarian cancer patients to a taxol-based regimen as a function of survivin expression, we found significantly higher clinical or pathological response rates in cases with absent or low protein expression than in those expressing high levels of survivin (Zhang et al., 2005). The present study clearly demonstrated that Oxaliplatin suppresses the survivin expressed breast cancer cells by targeting apoptosis.

Survivin has been implicated in human cancer development and is known to be over expressed in a variety of human cancers. Positive regulation of survivin may provide a connection between cell cycle regulation and tumorigenesis or perhaps tumor maintenance (Fengzhi et al., 2006). The data acquired in the present investigation proved survivin function in segregation of chromosome is essential for the cancer cell cytokinesis and karyokinesis. In conclusion, our observation in this study shows that survivin support the survival and proliferation of cancer cells. Therefore the present work indicates the failure of cytokinesis due to down-regulation of survivin expression in selected cancer cell lines tend to form
multinucleated cells; hence survivin has to be investigated as an anti-cancer target protein (Endoh et al., 2005).

4.5. Conclusion

In conclusion, Survivin, a unique anti-apoptotic factor, plays an important role in cell cycle regulation. Numerous clinical studies have shown that survivin is markedly overexpressed in most common types of cancer, suggesting that transcriptional deregulation is a major mechanism involved in aberrant expression of survivin in cancers. Absence of survivin, certain euploid human cells suffer missegregation of chromosomes, abortive assembly of microtubules late in mitosis, failure of cytokinesis. We attribute the defects in mitosis and cytokinesis by disruption of the CPP complex might be due to the expression of survivin down-regulation. These findings are in contrast with reports about the role of survivin in cell survival, cytokinesis, and segregation of sister chromatids, centrosome duplication and the mitotic spindle. It appears that survivin is playing dual role viz., to both chromosome segregation and cytokinesis in various mammalian cells. It seems reasonable to suspect that abnormal expression or function of survivin might contribute to tumorigenesis and multinucleated condition which we observed in this study. Future studies will expand on these insights into fundamental mechanisms of action of survivin in cell division and cell survival; it will help in validating the survivin network as a viable target for rational development of cancer therapy in humans. Our results suggest that Oxaliplatin that potently inhibits survivin that may exert outstanding cytotoxic effects on cancer cells.