Chapter 1

INTRODUCTION

From past 3 decades, significant efforts and progress have been done to know the molecular basis of cancer. Cancer is clearly associated with an uncontrolled increase in cell number, alterations in mechanisms regulating new cell birth, or cell proliferation. Decreased rates of cell death (apoptosis) are also now known to contribute to certain types of cancer.

1.1. Capabilities of cancer cell: Brief overview

1.1.1. Cancer cells are independent of external growth signals

There is plenty of evidence that cancers display dysregulation/failure in several signaling pathways. Acquired growth signal (GS) autonomy to be clearly defined by studies. Three common molecular strategies for achieving autonomy are involving alteration of:

(i) Extracellular growth signals,

(ii) Trans-cellular transducers of those signals

(iii) Intracellular circuits that translate those signals into action

While most soluble mitogenic growth factors (GFs) are made by one cell type in order to stimulate proliferation of another, the process of heterotypic signaling, many cancer cells acquire the ability to synthesize GFs (Fedi et al., 1997). The production of PDGF (platelet-derived growth factor) and TGFa (tumor growth factor a) by

glioblastomas and sarcomas, respectively, are the examples of this mechanism (Fedi et al., 1997). Another common alteration is the up regulation of growth factor receptors. In particular, the pathway of the epidermal growth factor receptor EGFR is hyper activated in approximately 30% of all cancers (Kolch, 2002). GF receptors, carrying tyrosine kinase activities in their cytoplasmic domains, are overexpressed in many cancers (Fedi et al., 1997). Receptor overexpression may enable the cancer cell to become hyper-responsive to normal or low levels of GF that would not trigger proliferation (Fedi et al., 1997). The most common mechanism of acquired autonomy drive from alteration in the downstream cytoplasmic components that receive and process the signals send by ligand activated GF receptors. The SOS-Ras-Raf-MAPK cascade plays a central role and in about 25% of cancers structurally mutated Ras relay cell division signals constantly without stimulation by normal upstream regulators (Medema & bos, 1993).

1.1.2. Cancer cells become refractory to growth inhibitory signals

Within a normal tissue, multiple anti-proliferative signals operate to maintain cellular quiescence and tissue homeostasis. Incipient cancer cells must evade these anti-proliferative signals for proliferation. Many and perhaps all anti-proliferative signals are funneled through the retinoblastoma protein (pRb), p107 and p130. pRb (hypo phosphorylated state) blocks proliferation by altering the function of E2F transcription factors that control the expression of a number of genes required for progression from G1 into S phase (Weinberg, 1995). Disruption of the pRb pathway liberates E2Fs and thus allows cell proliferation. The pRb signaling circuit, as

governed by TGFb, can be disrupted in a variety of ways in different types of human tumors (Fynan and Reiss, 1993). TGFb responsiveness loosed by down regulation of TGFb receptors and dysfunctional receptors (Fynan and Reiss, 1993; Markowitz et al., 1995).

1.1.3. Evading apoptosis

There are several possible pathways that activate the apoptosis program in a cell. The most common mechanism involves the tumor suppressor protein p53, one of the most extensively studied mammalian proteins. The p53 tumor suppressor protein can elicit apoptosis by upregulating expression of proapoptotic Bax in response to sensing DNA damage. Bax in turn stimulates mitochondria to release cytochrome C which is a potent catalyst of apoptosis. Surely, the most commonly occurring loss of a proapoptotic regulator through mutation involves the p53 tumor suppressor gene. The resulting functional inactivation of the p53 protein is seen in more than 50% of human cancers (Harris, 1996). Additionally, the PI3 kinase–AKT/PKB pathway, which transmits anti-apoptotic survival signals, can be activated by extracellular factors such as IGF-1/2 or IL-3 (Evan and Littlewood, 1998), by intracellular signals emanating from Ras (Downward, 1998), or by loss of the pTEN tumor suppressor (Cantley and Neel, 1999).

1.1.4. Limitless replicative potential

Telomere maintenance is a key feature in virtually all cancer cells by one or the other mechanism, telomeres are maintained at a length above a critical threshold, and this in turn permits unlimited multiplication of descendant cells. These mechanisms seem

to be strongly suppressed in normal human cells to deny them unlimited replication. The role of telomerase in immortalizing cells can be demonstrated directly by ectopically expressing the enzyme in cells, where it can provide unlimited replicative potential onto a variety of normal early passage in vitro (Bodnar et al., 1998; Vaziri and Benchimol, 1998).

1.1.5. Angiogenesis, invasion and metastasis

The oxygen and nutrients supplied by the vasculature are crucial for cell function and survival. Angiogenesis-initiating signals are initiated by vascular endothelial growth factor (VEGF) and acidic and basic fibroblast growth factors (FGF1/2). Each binds to transmembrane tyrosine kinase receptors displayed by endothelial cells (Fedi et al., 1997; Veikkola and Alitalo 1999). Tumors appear to activate the angiogenic switch by changing the balance of angiogenesis inducers and countervailing inhibitors (Hanahan and Folkman, 1996). Many tumors evidence increased expression of VEGF and/or FGFs compared to their normal tissue counterparts. In others, expression of endogenous inhibitors such as thrombospondin-1 or b-interferon is downregulated (Singh et al., 1995; Volpert et al., 1997).

The capability for invasion and metastasis enables cancer cells to escape the primary tumor mass and colonize new terrain in the body where nutrients and space are not limiting. Cell–cell adhesion molecules (CAMs) mediate cell-to-cell interactions and integrins which link cells to extracellular matrix substrates. All of these “adherence” interactions convey regulatory signals to the cell (Aplin et al., 1998). Changes in expression of CAMs in the immunoglobulin superfamily also

appear to play critical roles in the processes of invasion and metastasis (Johnson, 1991).

The second general parameter of the invasive and metastatic capability involves extracellular proteases (Coussens and Werb, 1996; Chambers and Matrisian, 1997). Protease genes are upregulated, protease inhibitor genes are down regulated, and inactive zymogen forms of proteases are converted into active enzymes. Matrix-degrading proteases are characteristically associated with the cell surface, by synthesis with a transmembrane domain, binding to specific protease receptors, or association with integrins (Werb, 1997, Stetler-Stevenson, 1999).

1.2. Natural polyphenols and preventive/protective role in cancer

The current demand for affordable therapeutics and more concerns about side effects of used drugs has drawn interest in phytochemicals and traditional medicines (Peet and Li, 1999; Naasani et al., 2003; Sarno et al., 2003). Plant secondary metabolites extractable as natural products from fruits, vegetables, teas, spices, and traditional medicinal herbs have identified various bioactive polyphenols that regulate multiple cancer-inflammation pathways. These compounds are cost effective, exhibit low toxicity and are readily available (Jin et al., 2010; Chen et al., 2009; Nieman et al., 2009). More recently, evidences showed that specific combinations of polyphenols may be more effective in protecting against cancer (Naasani et al., 2003; Olthof et al., 2000). The cancer preventive activities of these natural products are due to their effects such as induction of anti-inflammatory and antitumor or anti metastasis

responses by targeting specific key proteins (enzymes, receptors, transcription factors and others) involved in cancer progression.

Flavonoids belong to a group of polyphenolic compounds, which are classified as flavonols, flavonones, flavones, flavanols, flavan-3-ols and isoflavones according to the substitute positions present on the parent molecule. Flavonoids of different classes known to have several pharmacological activities. Flavonoids have also been known to possess biochemical effects, which inhibit a number of enzymes such as aldose reductase, xanthine oxidase, phosphodiesterase, Ca^{+2}-ATPase, lipoxygenase, cycloxygenase, *etc.* In view of their wide pharmacological and biological actions, they seem to be having a great therapeutic potential.

The aim of present research work is to explore the potential of quercetin and taxifolin towards the inhibition of enzymes, receptors, protein-protein complex *etc* which play a crucial role in cancer progression by using *in-silico* approach such as molecular docking and molecular dynamics simulation along with *in-vitro* effect of quercetin and taxifolin on the cancer cell line in isolated and combination form.