Introduction

Cancer

Cancer is a succession of genetic changes that transform a normal cell into a malignant one with acquired properties of rapid proliferation, invasion and metastasis. It is a multi-step process that includes transformation, growth promotion, tumor formation and malignant progression. During cancer progression, in the manifestation of the cancer phenotype, a large number of genes, molecules and pathways contribute to the transformation and subsequent malignancy of the cells. These molecules are involved in the loss of control of proliferation and deregulation of apoptosis, producing an excess of cells and forming a mass (tumor). The gain-of-function mutation of genes involved in proliferation and loss-of-function mutation of tumor suppressor genes both lead to excessive proliferative signals (Lowe et al., 2004).

Once the mutations cause genetic alterations in the cells (initiation), the altered cells progress through different stages, which includes 'hyperplasia,' wherein the altered cells divide in an uncontrolled manner and lead to excessive number of cells in that region, though they exhibit a normal phenotype. Further genetic changes in the hyperplastic cells lead to phenotype alteration due to which cells become more disorganized and start abnormal proliferation and this stage of cell progression is called 'Dysplasia.' When the additional changes make the cells and tissues appear even more abnormal and start spreading over a larger area of the tissue involved, primarily containing the altered cells, called 'Carcinoma *in situ.'* The cells often become more primitive in their capabilities, i.e., they become de-differentiated or anaplastic. Till this stage, the cells are contained within the initial location (*in situ*) and do not circumvent - the basal lamina for invasion. The tumor mass of this type is often cured by surgery since the abnormal cells are all in one location. Later, the carcinoma cells reach stage four, called Cancer (malignant tumors) and at this stage, tumors gain the ability to invade the surrounding tissues and spread (metastasize) to areas nearby the tissue of origin. These metastatic tumors are the most dangerous and account for a large percentage (approximately 90%) of cancer deaths (Sporn, 1996).

According to Hanahan and Weinberg (2000), there are six hallmarks of cancer *viz.,* self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis.

Self-sufficiency in growth signals

The normal cells require mitogenic growth signals, hormones and other growth regulators, which act as signaling molecules that are utilized by the cells to grow and divide. However, in cancer, these cells modulate the signaling mechanism in such a way that it can receive a constitutive growth signal for achieving limitless cell proliferation and growth. For this, most cancer cells either mutate the growth factor receptors or abolish certain signals such as tumor suppressors that prevent the growth of tumors. Sometimes these cancer cells convert most of the signaling pathways to become autocrine, i.e., they start synthesizing their own growth regulators to invoke their receptors thus they do not depend on other cells to generate regulators which act in the paracrine manner (Hanahan and Weinberg, 2000). They modulate all the major signaling pathways responsible for growth and proliferation. One such pathway is the PI3K/AKT signaling pathway, which is central to the growth, proliferation and survival of normal as well as the tumor cells. In most of the cancers, this pathway was utilized by the cells for their growth and prevention of death through mutation of the PTEN (loss of function), AKT (gain of function), or by receptor modulations (Akca et al., 2011).

PI3K/AKT signaling pathway

The phosphoinositide 3-kinase (PI3K) signaling pathway is a complex and tightly regulated network that is critical for many physiological processes such as cell growth, proliferation, metabolism and survival. Aberrant activation of this pathway can occur through mutation of almost any of its major nodes and has been caught up in several human diseases, including cancer. PI3K is a lipid kinase that phosphorylates the 3-hydroxyl group of phosphoinositides, generating second messengers that regulate several downstream pathways involved in normal physiology as well as in the diseased state. In mammals, three classes of PI3Ks have been reported that differ in their structure and substrate specificity. The class IA PI3Ks have been implicated in the etiology of various diseases including cancer. It is a heterodimer of a p110α catalytic subunit and a p85/55 regulatory subunit which is activated downstream of RTKs (receptor tyrosine kinases). If recruited to RTKs at the plasma membrane as a heterodimer and upon growth factor stimulation, the SH2 domain (Src oncogene homology-2 domain) of the p85 subunit binds to phosphorylated tyrosine in receptor tyrosine kinases which relieve inhibition of catalytic subunit and mediate recruitment of p110α to the plasma membrane (Ligresti et al., 2009). In unstimulated cells, the regulatory subunit p85/55 maintains the p110α catalytic subunit in a non-activated state. Upon activation, in stimulated cells, p110α catalytic subunit leads to the production of phosphatidylinositol 3,4,5-triphosphate (PIP3). The phosphorylated PIP3 recruits

AKT - a crucial protein containing a PH domain. When AKT is phosphorylated via PDK1 at Thr308 and Ser473 (Hafsy et al., 2012), it gets activated. Activated AKT has a key role in the regulation of downstream signaling components such as NF-κB, GSK-3β, mTOR, MDM2, BAD and p27 to mediate several functions, including cell cycle progression, survival, apoptosis, metabolism, protein translation and cell motility (Arcaro and Guerreiro, 2007, Manning and Cantley, 2007, Los et al., 2009). NF-κB - a downstream mediator of PI3K/AKT signaling pathway, is reported to be a constant active transcription factor in malignant lung cancer cells and induces the transcription of target genes to mediate EMT, invasion, angiogenesis, metastasis, proliferation and also prevents apoptosis (Baldwin, 1996, Aggarwal, 2004, Akca et al., 2011, Yun et al., 2013). NF-κB is located in the cytoplasm and its inhibitor kappa B (Iκ-B) protein maintains it in an inactive state. PI3K/AKT signaling pathway upon activation by any internal or external stimuli, leads to activation of IKKα, which phosphorylates Iκ-B proteins and exposes it for proteasomal degradation, which causes nuclear translocation and transcriptional activation of NF-κB (Lin et al., 1995, Karin, 1999). Reports also indicate that NF-κB could regulate or activate lung cancer invasion by PTEN inactivation through the PI3K/AKT/NF-κB pathway (Acka et al., 2011, Hu et al., 2014).

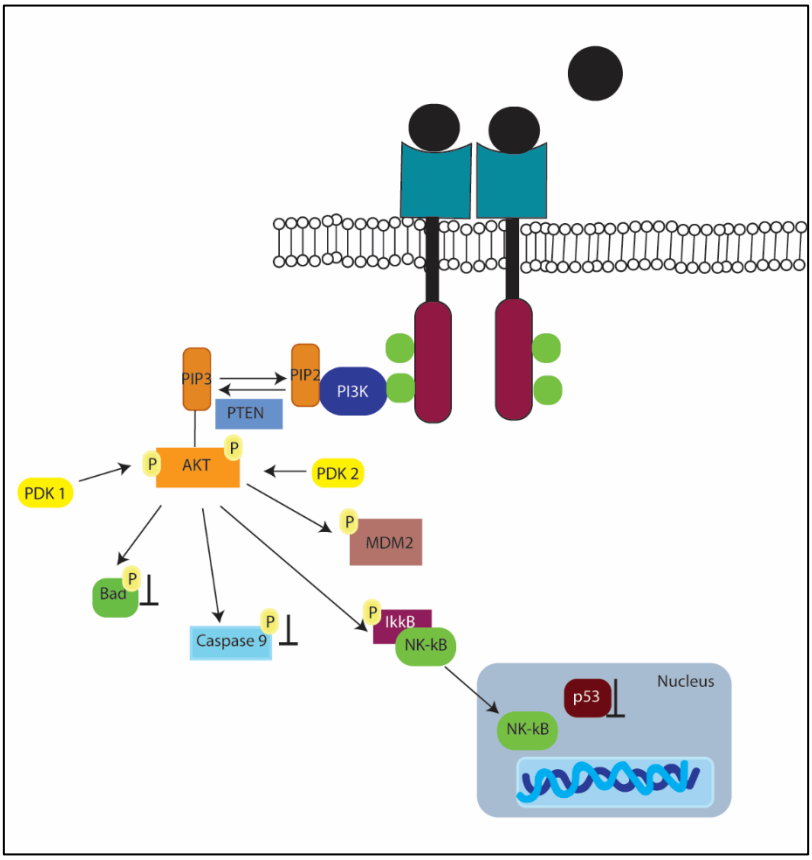

Figure i. Role of PI3K/AKT pathway in Apoptosis (Adapted from Kalimuthu et al., 2013)

AKT - a positive regulator of PI3K signaling, has also been reported to have direct interference with cell death pathways without any entwining mediators including NF-κB. For achieving its goal, AKT phosphorylates the key proteins regulating apoptosis and shifts the ratio of pro- and anti-apoptotic proteins towards inhibition of cell death. It also suppresses cellular stress-mediated apoptosis through removal of reactive oxygen species by increasing the expression of MnSOD (Ju et al., 2007). The activated AKT, phosphorylates and inhibits BAX and BAD, two key pro-apoptotic proteins of the BCL-2 family. AKT phosphorylates BAD on Ser136, which makes BAD dissociate from the BCL-2/BCL-X complex and lose the pro-apoptotic function. The activated AKT also phosphorylated the multi-domain BCL-2 protein BAX at ser184 residue to terminate its pro-apoptotic function. AKT also inhibits caspase mediated apoptosis via NF-κB mediated transcriptional activation of pro-survival gene BCL-XL and the X-linked inhibitor of apoptosis protein (XIAP), which binds and inhibits caspases and eventually apoptosis (Yamaguchi and Wang 2001, Gardai et al., 2004). AKT also phosphorylates MDM2 (murine double minute protein), an inhibitor of p53 protein, which, upon activation, suppresses the p53 activity (Figure i) (Kaltschmidt et al., 2000).

The activated PI3K/AKT signaling also contributes to metastasis and invasion during cancer progression. It is NF-κB, which, when activated through AKT, upregulates the downstream targets Twist1, Slug and Zeb2 - transcription factors responsible for Epithelial to mesenchymal transitions (Kumar et al., 2013) and MMPs (MMP-2 and MMP-9 expressions). Further, NF-κB maintains the level of AKT during cancer by downregulating the expression of PTEN -a negative regulator of AKT (Kim and Lee, 2005).

The PI3K/AKT signaling pathway also supports tumor angiogenesis - the process of sprouting of new blood vasculature from a pre-existing one or by inserting interstitial tissue columns into the lumen of pre-existing vessels (Carmeliet and Jain, 2000). This process is triggered by growth factor(s) binding or by genetic alterations that lead to activation of PI3K or by mutations of tumor suppressor genes such as PTEN and p53 (Folkman, 1995, Carmeliet and Jain, 2000). Among all the pro-angiogenic factors, vascular endothelial growth factor (VEGF) and angiopoietins play a significant role during tumor growth and angiogenesis. Among all the VEGFs (VEGF-A, -B, -C, -D), VEGF-α plays a crucial role in vascularization and angiogenesis. It (VEGF- α) binds to both VEGFR1 and VEGFR2 to regulate tumorigenesis and angiogenesis (Brown et al., 2001, Murakami et al., 2008). VEGFR1 binds to the p85 regulatory subunit of PI3K on Tyr1213 and 1333 and maintains a cross-talk with VEGFR2 for the regulation of cell migration,

differentiation, angiogenesis (Cunningham et al., 1995, Autiero et al., 2003) and proliferation of endothelial cells (Dayanir et al., 2001). It was noticed that when PI3K inhibitors, wortmannin and LY294002 were given, the VEGF-induced endothelial cell survival was blocked by overexpression of a dominant-negative form of AKT (AKT-DN) (Gerber et al., 1998).

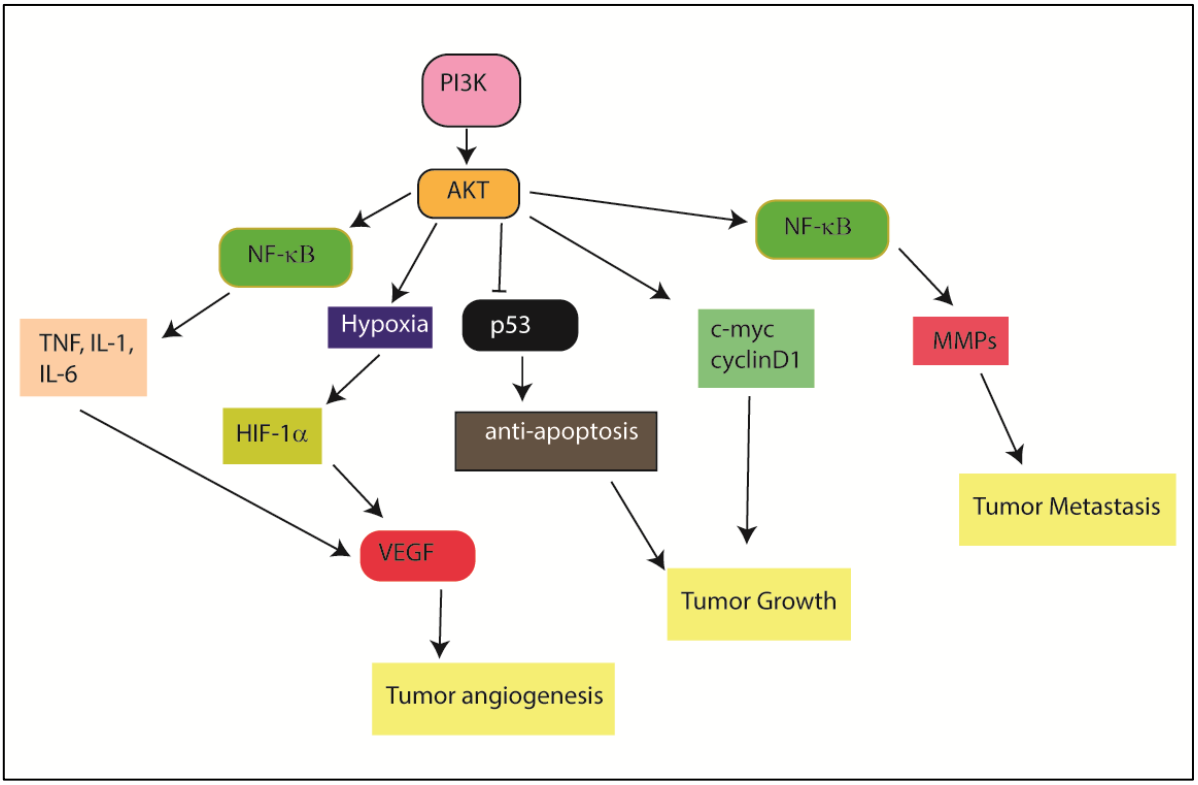

Figure ii. PI3K/AKT pathway in tumor cell growth, metastasis and angiogenesis

Since PI3K/AKT signaling is involved in all the steps of tumor growth, angiogenesis and metastasis (Figure ii), cancer cells utilize (upregulating), this pathway for achieving self-sufficiency in the growth and proliferation. Hence, many inhibitors for this pathway have been developed and some of them are in clinical trials showing promising anticancer effects and might emerge as drug of choice in the time to come. Any molecule which has the potential to combat the effect of the PI3K/AKT pathway can be a good candidate for anticancer chemotherapeutics development.

Insensitivity to anti-growth signals

Cancer cells acquire the capability to become resistant to the anti-growth signals, i.e., cell cycle arrest signals. Insensitivity to anti-growth signals and loss of tumor suppressors are the central hallmarks for cells to gain oncogenic property. During normal cellular growth and proliferation, check-point proteins and tumor suppressor proteins such as pRb, p53, PTEN etc. ensure that the cell is ready for division, as well as progression to the next phase of the cell cycle. Through these

tumor suppressor proteins, cancer cells target the cell cycle entry (pRb), cell cycle progression (p53) and human telomerase reverse transcription (hTERT) activation (Lundberg et al., 2002). Any abnormality like DNA damage, flaws during DNA replication, or segregation process, is sensed by the tumor suppressor proteins which eventually halts the cell cycle and activates the repair mechanism. However, when damage is very intense, these tumor suppressor proteins force the cells to abort the cell cycle and activate the mechanism of programmed cell death, i.e., apoptosis. In this way, these tumor suppressor proteins block the transformation of a normal cells to cancerous cells. Through mutating their functions, neoplastic cells attain the unlimited replicative potential and evade the cell cycle arrest, growth arrest and senescence by tumor suppressors. Dozens of tumor suppressor genes get activated to inhibit the proliferation of damaged/mutated cells by arresting cell cycle progression and inducing programmed cell death, hence, the evasion of these tumor suppressors is critical for carcinogenesis. p53 and Rb are typical tumor suppressor genes (Kohno and Yokota, 1999). Here in the study, the tumor suppressor protein p53 is focused.

p53

p53 is a tumor suppressor protein that has been known as a "guardian of the genome" because when cells are exposed to stress, like hypoxia, DNA damage and loss of normal cell contacts, it induces the senescence, cell cycle arrest or apoptosis (Fridman and Lowe, 2003). The importance of p53 can be understood by the fact that in more than 50% of human cancers, the functional inactivation of p53 protein, a product of the p53 gene is reported, which leads to the desensitization of DNA damage sensors that induce apoptotic cascades (Harris, 1996). TP53 is a nuclear DNA binding phosphoprotein that exists as a tetramer, which binds to a specific sequence of the DNA, (promoter region) and activates the target genes. MDM2 is an oncoprotein and a negative regulator of the p53. In fact, p53 and MDM2 proteins are maintained in a balanced situation in a normal cell. The loss of this balance (either inactivation of p53 or overactivation of MDM2) leads to carcinogenic phenotype. The tumor suppressor TP53 regulates several processes, e.g., cell cycle arrest, repair, senescence and apoptosis by transactivating the genes involved in these processes. These genes are *p21, MDM2, PCNA, cyclin D1, TGF-α, cyclin G, BAX, BCL-XL, Fas1, FasL DR-5 and Igf-BP3* (Beckerman and Prives, 2010).

The activation of p53 is induced by DNA damage, oncogenic as well as hyperproliferative stimuli (Ras, Myc and E1A), nucleotide deprivation, or in the presence of chemotherapeutics (Levine, 1997). When these stimuli are sensed by p53, it arrests the cell at the G1 phase of the

cell cycle by activating the expression of p21 protein (CKI-CDK inhibitor), which consequently decreases the cyclin D1/CDKs complex. In these circumstances, the phosphorylation of pRb was checked, which prevent the progression of cells from G1 to S phase. It also prevents re-replication when spindle fibers are damaged and hence, annuls the entry of cells into the S phase of the cell cycle (Di-Leonardo et al., 1997). Along with the cytostatic activity, p53 also activates the apoptosis in the presence of hypoxia, DNA damage, growth factor withdrawal and in the presence of Myc or E1A (Levine, 1997). Upon induction of apoptosis, p53 transactivates its downstream targets BAX, DR-5 and Fas to activate the process. Fas is a cell surface receptor, which upon binding of FasL (Fas ligand), triggers the extrinsic pathway of apoptosis (Figure iii) (Owen-Schaub et al., 1995). In the presence of DNA damage, p53 also transactivates DR-5 (similar to Fas) to stimulate caspase-dependent apoptotic response in the cell by binding to the ligand TRAIL (Wu et al., 1997). The choice to arrest cell cycle or induce apoptosis depends on the cell type and the intensity of the damage. If the cell is a null mutant to p21 or the cross-talk between the pRb and p53 is impaired, also if the damage is beyond repair, then p53 induces apoptosis. (Chiou et al., 1994, Polyak et al., 1996). However, the apoptotic effect of p53 can be reverted by pRB and MDM2 as both these molecules are caspase substrates. When apoptosis is activated, it cleaves the pRb and impairs the binding of MDM2 to p53. Nevertheless, it can associate and form a complex which directly blocks the E2F and p53 induced pathway of apoptosis (Janicke et al., 1996).

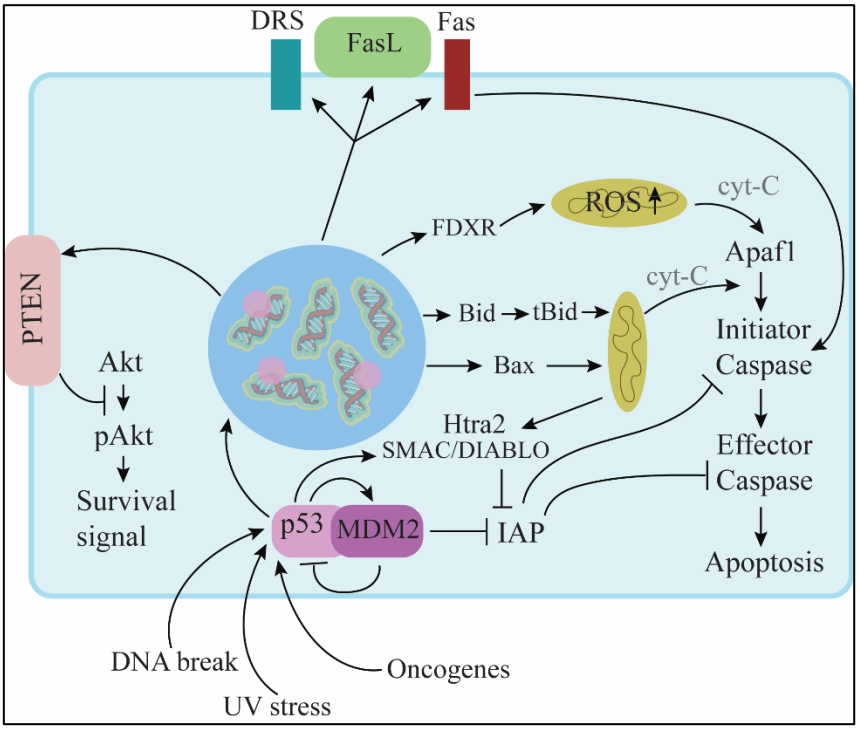

Figure iii. Apoptosis pathway induced by p53 (Adapted from Fridman and Lowe, 2003)

Loss of p53 not only helps in tumor initiation and progression but it also allows the tumor to acquire a full repertoire of metastasis quickly (Figure iv). It suppresses metastasis, in part, by negatively regulating the factors that initiate and maintain the EMT (epithelial to mesenchymal transition) process. To inhibit the process of EMT, p53 regulates the expression of MDM2, which in turn, degrades Slug to enhance E-cadherin expression and maintain the epithelial-like characteristics (Wang et al., 2009a). Snail and Slug are the master regulators behind the process of EMT. Twist, which is another transcription factor involved in the EMT process when gets activated it downregulates the ARF tumor suppressor protein, leading to the ubiquitin-mediated proteolysis of p53 via MDM2 (Maestro et al., 1999). p53 also suppresses invasion and angiogenesis by NF-κB mediated downregulation of MMP-9 and activation of ECM component thrombospondin (Liu et al., 2006). Therefore, tumor cells mutate or suppress TP53 function to attain full-fledged metastatic growth and due to this, it is the primary target for the medicinal chemist to design a molecule that can combat its loss or revert its function.

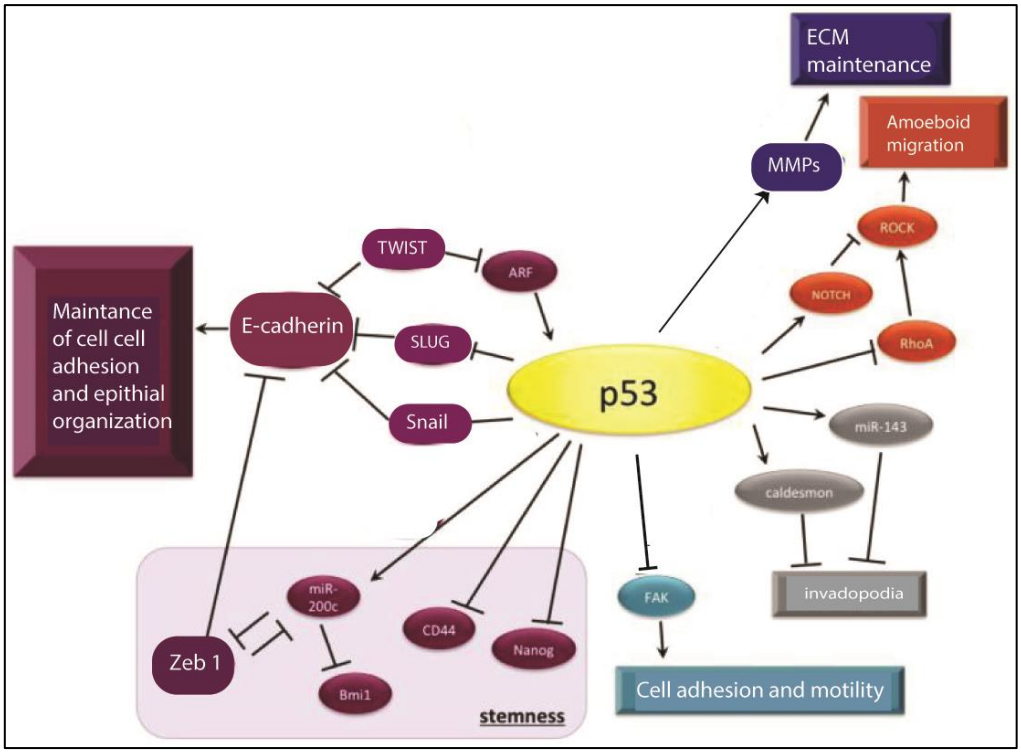

Figure iv. p53 a major contributor to tumor invasion and metastasis (Adapted from Powell et al., 2014)

Evading apoptosis

The expansion of a tumor does not only depend on the rate of cell proliferation but also the rate of cell attrition. Apoptosis is a programmed cell death in which cells die through a mechanism programmed in their genetics. It is a major source of cell death in an organism that is required

for the maintenance of the homeostatic balance of the body. Once the cell gets a physiological response for cell death, the mechanism starts in a well-choreographed manner. It includes cellular membrane disruption, nuclear and cytoplasmic skeleton breakdown, extrusion of cytosol, fragmentation of the nucleus, DNA damage and lastly the shriveled cells are engulfed by the nearby phagocytic cells without any damage to the surrounding healthy cells (Wyllie et al., 1980).

The apoptotic machinery is divided into two portions, first is a 'sensor', which senses the extracellular, as well as intracellular environmental changes and takes the decision over die or survive. The second is the 'effector' which includes all the signaling receptors that bind to the survival or the death factors, for example, the survival signal is conveyed by IGF-1/2 through their receptor IGF-R and by IL-3 through IL-3R (Lotem et al., 1996, Butt et al., 1999) and death signal conveyed by Fas ligand via binding to Fas receptor or by TNF-α binding to TNF-R1 (Ashkenazi and Dixit, 1999). The intracellular sensors monitor the intracellular abnormalities, including DNA damage, oncogenic provocation of signaling imbalance, survival factor deficiency, or hypoxic condition and activates the death pathway (Evan and Littlewood, 1998). This pathway is also known as the intrinsic pathway of apoptosis and is governed by mitochondria, which releases cytochrome c in response to the death signals (Green and Reed, 1998). In response to cytochrome c release, the BCL-2 family of pro-apoptotic proteins (BAX, BAD, Bid, BAK) or anti-apoptotic proteins (BCL-2, BCL-2W, BCL-2XL) get activated to perform their functions (Green, 2000).

As mentioned previously, in response to DNA damage p53 - a tumor suppressor gene elicits apoptosis by upregulating BAX - a pro-apoptotic member of the BCL-2 family, which in turn contributes to the cytochrome c release from the mitochondria. The released cytochrome c activates the ultimate effector of apoptosis, which is an array of proteases termed 'Caspases.' There are two types of caspases, 'gatekeeper caspases' and 'effector caspases.' Gatekeeper caspases are caspase 8 and caspase 9, which are invoked by death receptor Fas and cytochrome c of mitochondria, respectively. These proximal caspases (gatekeeper caspases) activate dozens of effector caspases that execute the death program through selective disruption of cellular components, subcellular organelles and the cell's genome (Thornberry and Lazebnik, 1998).

In 1972, for the first time, it was noticed that apoptosis could serve as a barrier in cancer growth when Kerr, Willie and Currie observed massive cell death (apoptosis) upon withdrawal of the

hormone in hormone-dependent tumor (Kerr et al., 1972). Certain events such as mutation in BCL-2 oncogene via chromosomal translocation in follicular lymphoma, c-Myc overexpression along with BCL-2 disruption, the Fas death circuit confirmed that cancer cells take advantage of these oncogenes to evade apoptosis (Korsmeyer, 1992, Hueber et al., 1997). Resistance to apoptosis is also acquired by cells through inactivation or loss of tumor suppressor genes functioning along with overactivation of oncogenes. The most commonly reported loss of pro-apoptotic regulation is through mutations involving the loss of tumor suppressor gene p53. Not only DNA damage but abnormalities induced by hypoxia and oncogenic hyperactivation, are funneled as a part of p53 to induce the apoptotic machinery and the loss of p53 function impairs the apoptotic mechanism to elicit a response (Levine, 1997). The PI3K/AKT signaling pathway also transmits the anti-apoptotic signals for survival and is involved in the mitigation of apoptosis in many human cancers. The survival signal can be abolished by curtailing the extracellular stimuli, eg. IL-3, IGF-1/2, through intracellular emanation from Ras or due to loss of PTEN a tumor suppressor phospholipid phosphatase and a negative regulator of AKT's survival signal (Cantlay and Neel, 1999).

However, most of the effector and regulatory components of apoptosis are present in a redundant form. Through utilization of an alternative pathway, new anticancer therapy can be developed, which can work even in the presence of a mutated apoptotic pathway.

Limitless replicative potential

When a cell population progress through a certain doubling number called 'Hayflick limit' (mostly 60-70 doubling in mammalian culture), it stops growing and reaches a stage called senescence (unable to divide but still alive). However, cultured fibroblast cells were provoked to divide for additional generations by disabling their pRb and p53 tumor suppressor proteins until they reach a crisis (die). The state of crisis is represented by massive cell death with karyotypic disarray, which is produced due to end-to-end fusion of chromosomes whereas, some cells (1 in 10^7 cells) get immortalized and acquire the ability to multiply limitlessly (Wright et al., 1989). This phenomenon of the cell to attain immortalization in the extreme condition of crisis suggests that under *in vivo* conditions tumor cells also try to attain a similar condition of crisis by enduring it as an essential part of the multi-step process of malignant transformation (Hayflick, 1997). The stage of senescence is attained preferably, due to telomere DNA shortening. Cancer cells overcome this barrier by manipulating telomerase (enzyme) to maintain the telomere length. Thus, they can divide indefinitely without initiating the process of senescence. To attain a

limitless proliferation, cancer cells maintain their telomere length by upregulating the expression of telomerase enzyme or by activating the mechanism of ALT which is virtually evident in all types of malignant cells (Shay and Bacchetti, 1997). Actually, senescence is a protective mechanism like apoptosis, which can be activated by shortening of telomere or in the presence of conflicting growth signals that prevent further cell proliferation. *In vivo* senescence represents an essential step during tumor progression, which is required for the breaching of the crisis barrier. Therefore, prevention at the stage of senescence can be a good target for therapy which will prevent further increase in the immortalization process during tumor progression.

Angiogenesis

Another hallmark of cancer progression and development is 'angiogenesis' (also known as neovascularization). It is the process of development of new blood vessels from the existing ones (Semenza, 2007). Angiogenesis is a complex process with many cell types, wherein soluble stimulators, inhibitors, the local matrix, inflammatory mediators, immune cells, as well as the tumor cell itself, act together in concert to determine the angiogenic response (Sozzani et al., 2007, Ramjaun and Hodivala-Dike, 2009). According to Folkman (1971), angiogenesis is the main contributor to tumor metastasis. Since, all carcinomas originate from the transformation of the normal epithelial cells, the unrestricted proliferation of these cells leads to formation of a mass of cells in the epithelial compartment, which either protrude toward the epidermis (out from the surface), into the lumen or filling the lumen of the glands. This stage is representative of "carcinoma *in situ*" or intraepithelial neoplasia and is considered as a precancerous neoplasm. However, for growth and proliferation, nutrient as well as oxygen supply are crucial and for that every cell will remain in the close proximity (within 100µm) of the blood vessel. Because of this dependency, every cell within the tissue has an intrinsic ability to encourage the growth of vasculature. However, cells which are under rapid proliferation, initially lack an angiogenic ability hence, curtailing the potential expansion. Nonetheless, in order to attain a large size (beyond 25mm), neoplasm needs angiogenesis and to maintain the extensive angiogenesis process, it is very important to maintain a balance among the cytokines, growth factors and inhibiting factors that regulate the functioning of endothelial cells (Folkman, 1997). The pro-angiogenic agents include VEGFs (vascular endothelial growth factor) and FGFs (fibroblast growth factor), epidermal growth factor (EGF), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), placental growth factor (PGF) and cytokines, such as tumor necrosis factor-α (TNF-α), colony-stimulating factor-1 (CSF-1) and interleukin-8 (IL-8) (Bergers and Benjamin, 2003, Amini et al., 2012, Simons et al., 2016). For attaining excessive

angiogenesis, angiogenic factors and cytokines are not only secreted by cancer cells themselves but also by the nearby cells such as endothelial cells, fibroblasts, platelets, smooth muscle cells and inflammatory cells (Ucuzian and Gassman, 2010). Other mediators of angiogenesis include bioactive lipids, such as prostaglandin E_2 (PGE_2) matrix degenerating enzymes, namely, matrix metallo proteases (MMPs) and heparinases and small mediators such as nitric oxide (NO), peroxynitrite, serotonin, as well as histamine, the angiopoietins (Ang) and erythropoietin (El-Kenawi and El-Remessy, 2013). In order to attract these angiogenesis-stimulating factors, cancer cells that are under hypoxia due to increased proliferation, stimulate the release of hypoxia-inducible factor-1α (HIF-1α), along with cytokines and anti-apoptotic proteins to provoke angiogenesis (Krock et al., 2011).

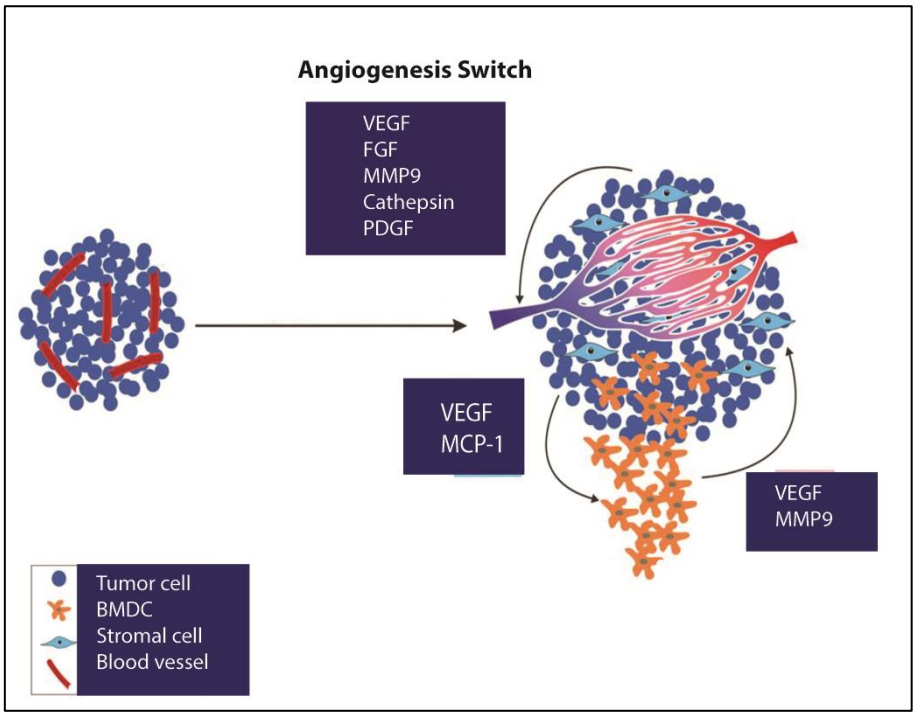

Figure v. Critical molecules involved in the angiogenic switch during cancer progression (Adapted from Saman et al., 2020)

The initiation of angiogenesis is a critical event that occurs during tumor progression via "angiogenic switch" from vascular quiescence (Figure v). This hallmark represents a shift from dormant to progressive growth. The tumor cells activate this angiogenic switch by changing the balance between the angiogenic inducers and their countervailing inhibitors. One most common strategy is to over-activate the VEGFs and FGFs expression (Singh et al., 1995). The VEGFs and FGFs act as initial angiogenic signals that bind to the tyrosine kinase receptors displayed by the endothelial cells (Veikkola and Alitalo, 1999).

VEGFs are the potent mitogens for the micro and macrovascular endothelial cells (Ferrara and David-Smith, 1997). It exists in five alternative splice variants namely VEGF121, VEGF145, VEGF165, VEGF189 and VEGF206 respectively (Houck et al.,1991). VEGF165, a 46-kD homodimeric glycoprotein, is the predominant isoform in normal and transformed cells and is present in a bound form in the extracellular matrix (Park et al.,1993). The promoter region of VEGF contains hypoxia-responsive elements indicating that hypoxia acts as a major mediator of angiogenesis via VEGF (Levy et al.,1995). It also acts as a paracrine mediator for indirectly acting angiogenic factors, such as TGF-β (Brogi et al., 1994). The tumor suppressor gene p53 in some cell types is known to positively regulate the negative inhibitor of angiogenesis namely, thrombospondin-1. Since in most of the cancers there is a loss of p53 expression which causes fall in thrombospondin-1 levels and hence, liberates endothelial cells from its inhibitory effect (Rak et al., 1995, Maxwell et al., 1999). Another mechanism through which tumor cells modulate angiogenesis is by activating proteases, such as MMPs. In the tumor microenvironment, TAMs (tumor-associated macrophages) deliver MMP-9, which releases VEGFs sequestered in the matrix. Free VEGFs stimulate vessel growth and motility. When the anti-VEGF antibody was used, it impaired the neovascularization and growth of subcutaneous tumors in mice (Kim et al., 1993), this result motivated the researches directed towards the importance of angiogenesis during tumor progression and the development of an anti-angiogenic therapy as a cure for cancer. Considering the importance of angiogenesis in the progression of tumor metastasis, investigating the utility of novel and potential anti-angiogenic compounds is of great importance to conquering the cancer. Therefore, tumor angiogenesis (indeed shared by perhaps all types of human tumors) is an attractive target for anticancer therapy.

Tumor invasion and metastasis.

During the tumor progression sooner or later the mass of cells invade adjacent tissue and travel to distant sites and settle in another niche. This distant settlement of tumor cells is known as metastasis and is a major cause of cancer-related death worldwide (Sporn, 1996). The invasion and metastasis enable these cells to settle down in a new terrain in the body where nutrients and oxygen are not limiting, at least initially. However, to attain invasive and metastatic ability, firstly the cancer cells change their phenotype as well as plasticity through Epithelial to mesenchymal transition in which cells lose their epithelial characteristics, attain migratory mesenchymal characteristics and start separating from tumor mass to invade the nearby tissue. Moreover, once the cells attain the mesenchymal characteristics, they upregulate the

extracellular proteases which enable the remodeling of ECM (Extracellular matrix) to invade the nearby tissue.

Epithelial-Mesenchymal Transition (EMT)

EMT is a highly conserved process that occurs during embryogenesis, chronic inflammation, as well as during cancer metastasis and is referred to as type I, type II and type III EMT, respectively (Kalluri and Weinberg, 2009). Molecularly, EMT is characterized by loss of epithelial characteristics and the concomitant gain of mesenchymal characteristics through the alteration of gene expression. Several proteins are modulated to attain the epithelial to mesenchymal transitions. One such protein is CAM, which includes members of immunoglobulins and calcium-dependent cadherins which mediate cell to cell interaction. However, the other proteins like integrins enable the cell to matrix attachment. Nonetheless, all these adhesions act as signals to the cells (Aplin et al., 1998). Customarily, epithelial cells are involved in the lining of the cavities and surfaces of tissues and organs and organizing themselves into sheets that are held together through several interactions, including tight junctions, adherens junctions, desmosomes and gap junctions. Epithelial cells are polarized in an apical-basal orientation and are tethered to neighboring cells through intercellular junctions that permit only cohesive epithelial cell movement. The measure of alteration observed in the cell environment is a change in the levels of E-cadherin, a ubiquitously expressed homotypic cell to cell interaction molecule, on the epithelial cells. Coupling of E-cadherin molecules present on the adjacent cells transmits the anti-growth signals via cytoplasmic contact with β-catenin to an intracellular circuit, which includes transcription factor Lef/Tcf (Cristofori and Semb, 1999). In most of the epithelial cancers, E-cadherin is lost either due to mutational inactivation of E-cadherin or β-catenin. Based on their study using a mouse model of carcinogenesis, Cristofori and Semb (1999) have opined that if E-cadherin is forced to express, the invasion and metastatic capability of epithelial cells will reduce appreciably. Thus E-cadherin functions as a widely acting suppressor of invasion and metastasis and its functional elimination will help in the acquisition of this capability. Downregulation of E-cadherin by the transcriptional repressors like Snail/SNAI1, Slug/SNAI2, IP1/ZEB2 or Twist leads to the disassembly of adherence junctions and translocation of membrane-bound β-catenin into the cell nucleus where it modulates transcription of numerous genes such as c-myc and cyclin D (Peinado et al., 2007). The subsequent upregulation of mesenchymal markers such as vimentin and neuronal (N) cadherin are reported to aid epithelial-mesenchymal transition (Yilmaz and Christofori, 2010). This change from E to N-cadherin expression, is termed as ‘cadherin-switch’ which leads to enhanced motility of EMT-transformed

cells. Thus, cells that have undergone EMT lose their epithelial characteristics, separate from the nearby cells and attain the ability to migrate (Figure vi).

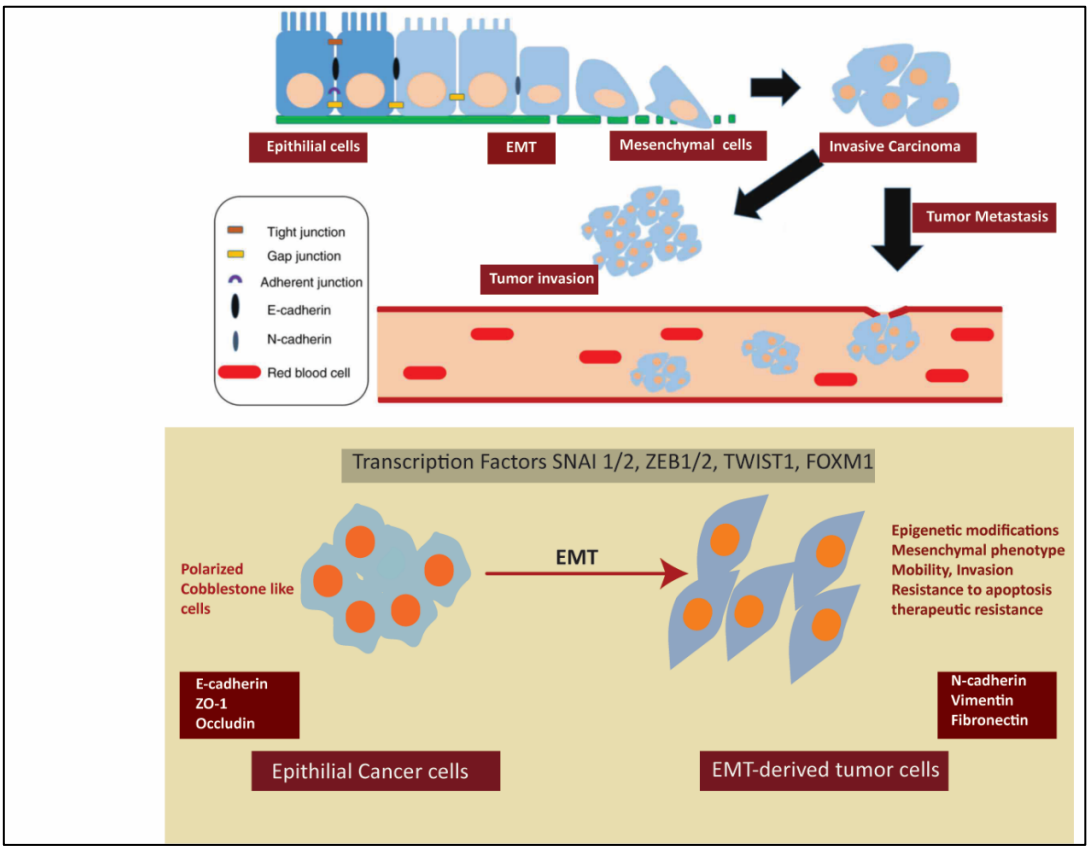

Figure vi The major cells and molecule involved in the EMT process during tumor invasion (Adapted from Li et al., 2019, Roche, 2018)

ECM remodeling and Matrix metallo proteases (MMPs)

The second general parameter for invasion and metastasis involves extracellular proteases such as matrix metallo proteases, which act as principal mediators of the cancer microenvironment during cancer progression (Chambers and Matrisian, 1997, Kessenbrock et al., 2010). MMPs belong to a zinc-dependent family of endopeptidases implicated in a variety of physiological processes, including wound healing, organogenesis, as well as in pathological conditions such as inflammatory, vascular and auto-immune disorders and have also been known to play a role in carcinogenesis (Nagase et al., 2006). The increased expression of MMP in several tumor microenvironments was observed. Cancer cells stimulate host cells such as fibroblasts to secrete MMPs through the secretion of interleukins and growth factors and direct signaling through extracellular MMP inducers. During metastasis, transformed mesenchymal cells need to invade the dense matrix, in which MMPs play a crucial role by mediating the degradation of ECM and generating a path for the migration of cancerous cells (Figure vii) (Kumar et al., 2016). The

proteolytic activity of MMPs is required for cancer cells not only to degrade physical barriers during local expansion and intravasation at nearby blood vessels but also for the extravasation and invasion at a distant location. During the invasion, the localization of MMPs to specialized cell surface structures, called 'invadopodia,' is required for their ability to promote invasion. These structures represent the site where active ECM degradation takes place. Invadopodia utilize transmembrane invadopodia-related proteases, including MMP-14 MMP-2 and MMP-9, to degrade a variety of ECM macromolecules and facilitate the cell invasion. These proteases not only help the cancer cells during the invasion but also act as a signal for angiogenesis, cell growth and differentiation (Egeblad et al., 2002). It also acts as an anti-apoptotic signal in the cancer cells, eg. MMP-7 cleaves Fas ligand and make the cancer cells resistant to chemotherapeutics that induce apoptosis (Mitsiades et al., 2001, Strand et al., 2004). Notably, MMPs may contribute to the anti-apoptotic effect by indirectly activating serine/threonine and AKT/PKB through the signaling cascades of EGFR and IGFR (Kulik et al., 1997, Gialeli et al., 2009).

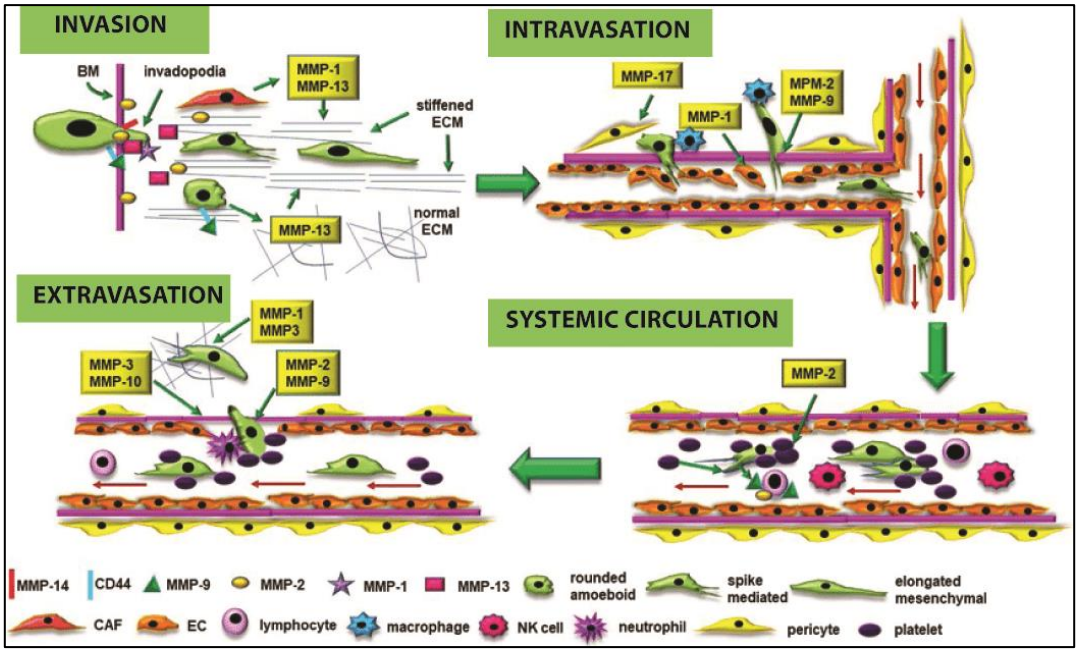

Figure vii. Role of MMPs in tumor metastasis (Adapted from Gonzalez-Avila et al., 2019)

MMPs also contribute to tumor angiogenesis, especially MMP-2, MMP-9 and MMP-14 and, to a lesser extent, MMP-1 and MMP-7 (Rundhaug, 2003). MMP-9 plays a significant role during the "angiogenic switch." It degrades extracellular components, such as collagen type IV, XVIII and perlecan for increasing the bioavailability of essential factors such as the VEGF and FGFs for the process of angiogenic switching (Bergers et al., 2000, Iozzo et al., 2009). During EMT, cell movement is highly dependent on the proteolytic activity of MMPs which are known for

regulating the dynamic ECM–cell and cell-cell interactions. Several integrins play an active role in the regulation of cell migration because they can serve as substrates for MMPs (Baciu et al., 2003). The communication between the cells is disrupted due to the shedding of E-cadherin. This morphological transition is achieved by MMP-1 and MMP-7, which contribute by cleaving E-cadherin (Noe et al., 2001). Studies have indicated the implication of MMP28 in the proteolytic activation of TGF-β, a powerful inducer of EMT and thereby helping in evading the immunosurveillance of cancer cells during circulation (Illman et al., 2006, Heldin et al., 2009). The host immune system is capable of recognizing and attacking cancer cells by recruiting tumor-specific T-lymphocytes (T-cell), Natural Killer cells (NK cells), neutrophils and macrophages. By contrast, cancer cells evolve escaping mechanisms using MMPs to acquire immunity. MMPs can increase TGF-β - a suppressor of T-lymphocyte against cancer cells (Gorelik and Flavell, 2001). MMPs, decrease cancer cell sensitivity to NK cells by generating a bioactive fragment from the protease inhibitor (Kataoka et al., 1999). Hence, MMPs are the crucial proteases involved not only in cancer invasion and metastasis but also in immunosurveillance during circulation, angiogenesis and cell death escape of the tumor cells. Therefore, several MMP inhibitors were developed in an attempt to control the synthesis, secretion, activation and enzymatic activity of MMPs. Natural compounds were also screened for their anti-protease activity as they pose less side-effects to adjacent cells. One such compound is neovastat, whose standardized extract when orally administrated exerted anti-angiogenic and anti-metastatic properties (Falardeau et al., 2001). Another natural agent that exerts its anticancer effects via inhibiting MMPs is genistein, a soy isoflavonoid structurally similar to estradiol (Zaczek et al., 2005).

Overall, despite all the advancements in its treatment, cancer is still a clinical challenge and requires novel multi-target agents to inhibit multiple signaling pathways involved in its progression. The discussed hallmarks uncover the critical pathways and the molecules required by the cancer cells to attain their limitless proliferation and metastasis. Through an understanding of these critical molecules, a potent and specific moiety can be designed by adding different pharmacophores which have the potential of targeting these critical molecules.

Lung cancer

Among all cancer types, lung cancer is the most deadly cancer in both men and women (Siegel et al., 2014). According to the American Cancer Society 2015, its death rate is the highest, exceeding the death rate of combined breast, colon and pancreatic cancer, which are the most commonly occurring cancers. It is responsible for around 1.4 billion deaths per year (Wood et al., 2015) The 5-year survival rate is approximately 17.8% and more than half of patients with lung cancer die within a year of diagnosis (SEER Cancer statistics review 1975-2011). Lung cancer is basically of two subtypes - small-cell lung carcinoma, which accounts for 15% and non-small cell lung carcinoma (NSCLC), which accounts for 85% of all lung cancers (Sher et al., 2008). NSCLC is further categorized into three sub-types namely, squamous-cell carcinoma, large-cell carcinoma and adenocarcinoma.

Squamous-cell carcinoma comprises 25-30% of all lung cancer cases and is strongly related to smoking. It arises from early squamous cells of the airway epithelial cells found in bronchial tubes (Kenfield et al., 2008). However, Large cell carcinoma accounts for 5–10% of the total lung cancers and is associated with smoking. It often begins in the central part of the lungs, near the lymph nodes and grows into the chest wall. Adenocarcinoma is reported to be the most common type of lung cancer (40% of all lung cancers diagnosed) however, since it grows slowly, it has a greater chance of being detected before it can spread out of the lungs. It arises from type II alveolar cells that secrete mucus (Noguchi et al., 1995) and is a commonly occurring lung cancer type both in smokers as well as in nonsmokers regardless of their gender and age (Couraud et al., 2012). It mostly occurs at the periphery of the lung, which might be due to the inhalation of large particles from filtered cigarettes that are unable to enter in the lungs.

After the emergence of the knowledge about NSCLC as a major health problem in the early 1970s, it was treated by pneumonectomy or lobectomy, which was solely a curative option and benefitted only a small number of patients who had a localized occurrence of the disease. However, no effective treatment was available for the patients who were under the advanced stage (Overholt, 1936). From the late 1980s, the platinum-based doublet chemotherapy came into picture, in which the cytotoxic drugs were added to the platinum backbone and it was used to cure advanced NSCLCs, which last for 25 years with minor changes in the drug composition and addition of more than one cytotoxic drug. However, the benefits were not consistent (Delbaldo et al., 2004, Paccagnella et al., 2006). In the 21st century, the use of cytotoxicity-based drugs became outdated and new approaches based on molecular biology or targeted therapy

arrived, which targeted the important molecules involved in the cellular pathways that regulate tumor growth and metastasis. The most important target for the development of anti-NSCLC drugs includes EGFR (epidermal growth factor receptor), which has been reported to be overexpressed in 70-80% of NSCLC. Moreover, increased gene copy number of EGFR was also reported in more than 60% of the tumors. The other targets consist of PI3K/AKT pathway and the RAS/RAF/MEK/MAPK pathway (Han et al., 2012). The second most targeted mechanism includes angiogenesis and the targeted molecules are VEGFs (Zhan et al., 2009).

Undoubtedly, radiotherapy and chemotherapy are also found effective against many cancers but pose serious and severe side-effects as well as complications (Qi et al., 2010). However, development of resistance to chemotherapy is very common in cancer, thereby, limiting the benefits (Safarzadeh et al., 2014). Apart from this, the available medication does not increase the life-span nor the quality of life for the lung cancer patients. Therefore, constant efforts are being made to develop novel medications so as to give maximum benefits to the lung cancer patients.

Plants are the richest source of natural compounds that exhibit medicinal properties and have been used for the treatment of various diseases for thousands of years. According to the WHO, approximately 80.0% of the world's population still depends on traditional medicines for their basic health care (Shoeb, 2006). Therefore, in search of a more potent and specific medication for NSCLC, the phytochemical 'Coumarin' was tried and the results revealed that some of its derivatives are quite effective in curtailing the cell proliferation (Belluti et al., 2010, Zhang et al., 2014a).

Coumarins

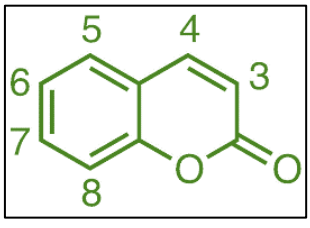

Figure viii. The structural framework of the coumarin ring (Adapted from Matos et al., 2015)

The phytochemical coumarin belongs to benzopyran (1,2-benzopyrones or 2H-1-benzopyran-2-ones) family of chemicals (Figure viii). They represent an important family of naturally occurring oxygen-containing heterocycles of the benzopyrone framework (Venugopala et al.,

2013). Actually, it belongs to a class of lactones structurally constructed with a benzene ring fused to an α-pyrone ring. The compound possesses a conjugated system with a rich supply of electrons and good charge-transport properties (Harborne 1982, Murray 1997). The name coumarin was derived from a French word 'coumarou,' a vernacular name of Tonka Beans, *Dipteryx odorata* (*Coumarouna odorata*), from which coumarin was first isolated in 1820. Coumarins are found throughout the plant kingdom ranging from essential oils (cinnamon bark oil, cassia leaf oil and lavender oil) and green tea to fruits (e.g., bilberry, cloudberry). Coumarins are divided into four sub-types: the simple coumarins, furanocoumarins, pyranocoumarins and the pyrone-substituted coumarins (Jain and Joshi, 2012). The simple coumarins include; coumarin, 7-hydroxycoumarin and 6,7-dihydroxy coumarin, these are hydroxylated, alkoxylated and alkylated derivatives of the parent coumarin molecule. In Furanocoumarins, the basic coumarin nucleus consists of a five-membered furan ring (Jain and Joshi, 2012). Similarly, Pyranocoumarin is analogous to the furanocoumarins, but it contains a six-membered ring instead of a five-membered ring. The well-known compound warfarin belongs to the pyrone substituted coumarin class. The other such compound includes 4-hydroxycoumarin which also has coumarin substituted pyrone ring (Kayal et al., 2014).

The coumarin compounds are reported to have a robust pharmacological activity, low toxicity, minimum side-effects, fewer instances of drug resistance and high bioavailability to treat various types of diseases (Wang et al., 2009). Even naturally occurring coumarins are also known for their wide spectrum of pharmacologically significant activities such as antioxidant (Kostova et al., 2011), anti-inflammatory (Fylaktakidou et al., 2004), antinociceptive (Peng et al., 2013), hepatoprotective (do Prado, et al., 2003), antithrombotic (Ostrov et al., 2007), antiviral (Emami et al., 2008), antimicrobial (Gormley et al., 1996), anti-tuberculosis (Manvar et al., 2011), anti-carcinogenic (Nair et al., 1991), antidepressant (Shashidhara et al., 2011), antihyperlipidemic (Yuce et al., 2009) anticholinesterase (Razvi et al., 2013) and anticancer (Alipour et al., 2014), activities. Hence, it has attracted the medicinal chemists to develop more effective medications by exploiting its basic properties.

In recent years, molecular hybridization strategy has emerged as a novel approach which involves the conglomeration of two or more pharmacophores in one molecular framework to develop hybrid multifunctional molecules (Ao et al., 2009). Many of the coumarin derivatives are hybrid molecules designed through the molecular hybridization technique by adding different pharmacophores on the basic coumarin ring. The mixing of pharmacophores in one molecular

scaffold makes the drug a candidate of paramount significance for the treatment of multifactorial diseases such as cancer (Emami et al., 2015). Various pharmacophores are designed in such a way that they can target multiple pathways and molecules at a time with reduced side-effects and with increased oral bioavailability and pharmacokinetic property. Hybridization of different coumarin derivatives with varied bioactive molecules such as resveratrol, maleimide sulfonamides, pyrazoline, chalcone, triazoles and lipoic acid has been used to develop novel hybrid molecules, which act as vasorelaxants (Campos-Toimil et al., 2002), platelet anti-aggregating (Singh and Pathak, 2016), anticancer (Klenkar and Molnar, 2015), antimicrobial, antioxidant and anti-inflammatory properties (Sandhu et al., 2014).

Coumarin as an anticancer drug

Anticancer drugs are mostly cytotoxic and are associated with severe side-effects, particularly for the naturally proliferating tissues such as the hematopoietic system. Often combination therapies have been preferred, wherein several cytotoxic agents are combined to treat a single type of cancer because it offers better results with fewer side-effects (Hanahan et al., 2000). Due to this, researchers are now exploiting the natural products having anticancer properties to develop drugs with minimal side-effects (Figure ix). As discussed previously, among various phytochemicals, coumarins have attracted the medicinal chemists in the past few years. Coumarins have the potential not only to treat cancer but also to combat the side-effects associated with conventional interventions like chemotherapy and radiotherapy (Finn et al., 2002). As demonstrated by Mahler and coworkers, administration of coumarin-troxerutin combination protected the salivary glands and mucosa of patients undergoing radiotherapy (Mahler et al., 1992). Coumarins are also known to inhibit cytochrome P450, thereby indirectly influencing the concentration and half-life of co-administered anticancer drugs in the blood (Foroozesh et al., 2019).

Apart from their use as a constituent of combination therapy, the natural coumarin derivative 7-hydroxycoumarin (the first metabolite) imposes strong cytotoxic activities against various cancer cell types, including A549 (human lung adenocarcinoma cell line) (Klenkar and Molnar, 2015). Similarly, 4-Arylcoumarin from the endophyte *Streptomyces aureofaciens* is a potent inhibitor of Lewis Lung Carcinoma (LLC) cell proliferation. Toddaculin, is a prenylated coumarin isolated from *Toddalia asiatica* also reported to have anti-neoplastic property against U-937 cell line with an IC_{50} of 51.38μM. The 3-substituted coumarin, Genistein - a natural component of

soy also belongs to the benzo-gama pyrones and has been intensively investigated as a chemo-preventive agent against hormonally regulated breast and prostate cancers in animal models (Constantinou et al., 1990, Finn et al., 2002). Not only natural but several synthetic coumarin derivatives were also designed based on the naturally occurring coumarins and they showed improved anticancer effects than their parent compounds such as the synthetic scopoletin derivatives which registered promising antitumor activities. In a recent study it was observed that, out of 20 synthesized derivatives of scopoletin tested, five compounds showed significant cytotoxicity (IC_{50}) at less than 2μM in MCF-7, MDA-MB 231 and HT29 cell line (Liu et al., 2012). Another derivative, 7,8 dihydroxy coumarin has inhibited the proliferation and induced the apoptosis in A549 human lung adenocarcinoma cells by suppression of AKT/NF-κB signaling pathways (Wang et al., 2013). Application of 3-(4-(2-(dimethylamino) ethoxy) phenyl)-7-methoxy-4-phenyl-2H-chromen-2-one (a synthetic coumarin derivative) induces the apoptosis by increasing the expression of pro-apoptotic BAX to BCL-2 ratio in the A549 cell line(Musa et al., 2012).

Both synthetic and natural coumarins not only show anticancer effects *in vitro* and *in vivo* through various mechanisms but have also been reported to inhibit angiogenesis a common phenomenon shared by all types of metastasized cancers (Avin et al., 2014, Thakur et al., 2015). Fundamentally, inhibition of angiogenesis is one of the most important anticancer mechanisms of secondary plant metabolites, including coumarins. Natural and synthetic coumarins with different structures can inhibit the factors involved in angiogenesis, migration, proliferation and differentiation of endothelial cells in *in vitro* as well as *in vivo* testing conditions. Coumarins act by blocking various molecular signaling pathways that include, growth factors (e.g., VEGFs, TNF-α and FGF-2), cytokines (e.g., IL-1 and IL-6), angiogenic enzymes (e.g., MMP), endothelium-specific receptor tyrosine kinases (e.g., Tie2) and adhesion molecules (e.g., intercellular adhesion molecule-1) (Nishida et al., 2006, Pan et al., 2011a, Sandhiutami et al., 2017). Scopoletin, isolated from *Erycibe obtusifolia* stem, when administered at a dose of 100nM significantly reduced the blood vessel branch points in chick chorioallantoic membrane. It also showed an inhibitory effect on the migration, proliferation and tube formation in the HUVECs (Human umbilical vein endothelial cells) (Pan et al., 2011b). The other coumarin derivative decursin and decursinol angelate, isolated from *Angelica gigas* root, also showed an anti-angiogenic effect on CAM by reducing the VEGFR and its downstream signaling molecules (Jung et al., 2009). Another natural coumarin, a furanocoumarin isolated from ethanolic extract of the twigs of *Broussonetia kazinoki*, exerted its anti-angiogenic effect by decreasing the levels

of MMP-2 and VEGF-α (Kim et al., 2015). VEGF-α stimulated various downstream molecules of angiogenesis signaling pathways, such as focal adhesion kinase (FAK), Src kinase, MEK, ERK, Akt and p70S6K. All these pathways were targeted by marmesin to check its anticancer effect. Moreover, reduction and blocking of VEGFs, MMPs and PI3K/AKT activity or function has reported to be among the most important anti-angiogenic and anti-metastatic mechanisms of coumarins such as galbanic acid (Kim et al., 2011), umbelliprenin (Alizadeh et al., 2018), imperatorin (Wang et al., 2017), auraptene (Jamialahmadi et al.,2018), esculetin (Park et al., 2016), osthole (Yao et al., 2018) and scopolin (Pan et al., 2009).

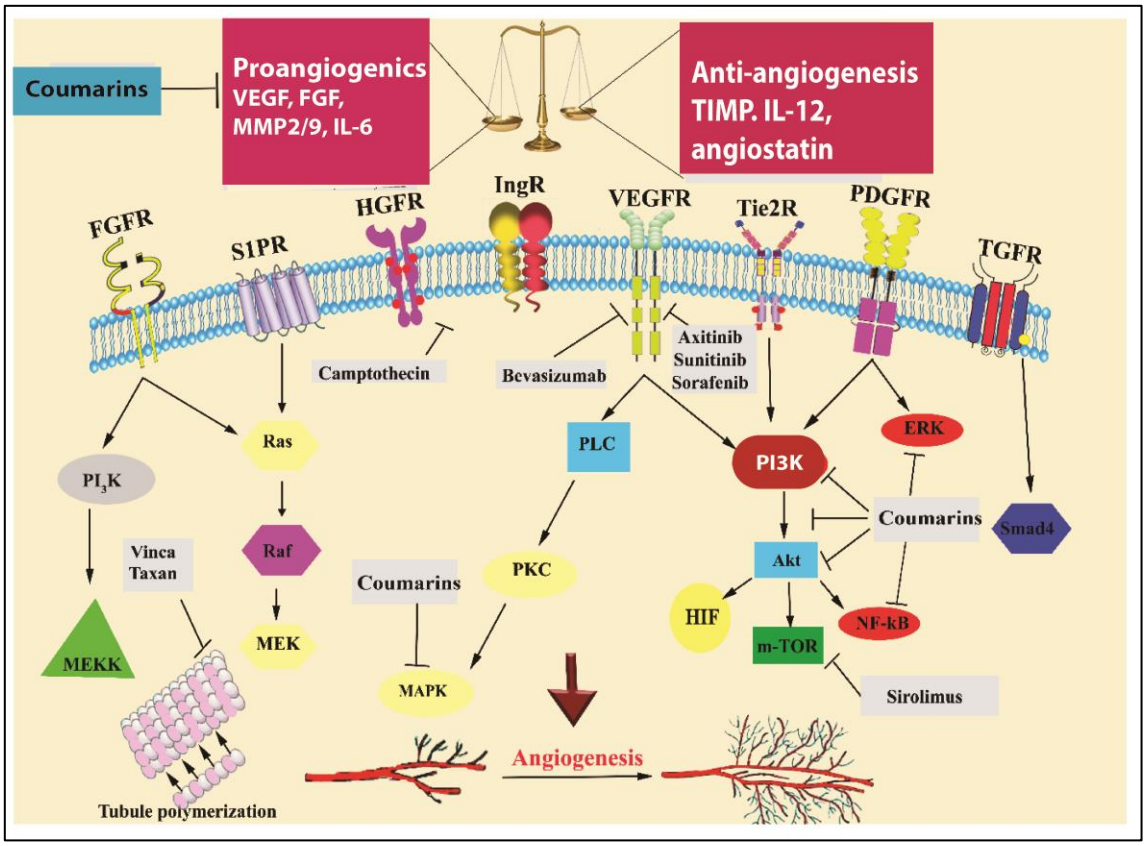

Figure ix Coumarin targeting the important nodes of cellular pathways involved in tumor growth, metastasis and angiogenesis (Adapted from Majnooni et al., 2019)

Origin of the problem

It is evident from the discussion above that natural coumarin derivatives exert minimal side effects while evoking effective anti-proliferative and anti-angiogenic properties compared to the conventional cytotoxic drugs which are used presently to treat the cancer. However, it was observed that amalgamation of other chemical moieties with the coumarin nucleus improves the anticancer property of the latter (Sandhu et al., 2014). This improvement in anti-proliferative activity can be attributed to the presence of different pharmacophores in a single molecule and therefore, can act on diverse regulatory and cellular pathways of cancer progression (which are discussed previously) at once to effectively curb the pathogenesis. However, as mentioned earlier, non-small cell lung carcinoma still remains as one of the most prevalent and deadliest form of cancers. The treatment regime followed today is hardly effective in combating this dreaded disease along with attended side effects that severely compromise the quality of life for the patients. Therefore, it was pertinent to find a safe yet effective alternate drug for the treatment of non-small cell lung carcinoma and hence, the idea of utilizing novel coumarin derivatives for this purpose was proposed.

Synthetic coumarin as an anticancer drug: Current status

Tabana and fellow workers (2016) have used scopoletin, a well-known natural coumarin with anti-angiogenic properties, to synthesize chemical analogs by adding various pharmacophores. A subsequent *in vitro* analysis, using human cancer cell lines, revealed that one of the synthetic coumarin derivatives, 4-[7-(diethylamino)- 2-imino-2H-chromen-3-yl]-6-(4-phenylpiperazin-1-yl)-1,3,5-triazin-2-amine, hampered the progression of cell cycle as well as angiogenesis more effectively than the rest of the derivatives as well as the parent compound (Tabana et al., 2016).

In another study, the efficacy of twenty-six new synthetic coumarins were tested against hepatocellular carcinoma cells and it was found that (7-carbethoxyamino-2-oxo-2H-chromen-4-yl)- methylpyrrolidine-1-carbodithioate is effective in inducing cytotoxicity at a very low concentration (Neelgundmath et al., 2015). They also reported that, the compound induced cytotoxicity by preventing the binding of NF-κB to DNA and hence, inhibiting the expression of genes such as cyclin D1, BCL-2, survivin, MMP12 and c-Myc which are known to influence cell proliferation and progression of tumor (Neelgundmath et al., 2015).

More recently it was reported that, the coumarin derivatives which possess N-aryl carboxamide-a phenyl substitution at the C-3 position and 1, 2, 3-triazolyl, trihydroxystilbene and amino substitutions at the C-4 position are effective in targeting lung cancer (Kumar et al., 2018).

Aim and objectives of the present study

The overall aim of the current study was to find a novel synthetic coumarin derivative that can effectively curtail the proliferation and migration of non-small cell lung carcinoma using A549 cell line - a widely accepted *in vitro* model for NSCLC.

In order to achieve this aim, utilizing the primary nucleus of coumarin, a series of eighteen novel synthetic derivatives were designed by adding various pharmacophores at the C-4 (mono-substitution) and C-3 and C-7 (di-substitution). The drug was designed by our collaborators from the synthetic organic chemistry laboratory of The Maharaja Sayajirao University of Baroda and therefore the details are not included here as a part of this thesis. However, their potential to target the critical pathways of cancer were evaluated using A549 human lung cancer cell line and was the focus of the current study. The above objective was achieved by five parallel studies, which can be treated as specific objectives, as listed below:

1. To screen the novel synthetic analogs of coumarin for their potential anticancer property in A549 cell line with concomitant evaluation for side-effects of the derivative showing lowest half-maximal inhibitory concentration in NIH3T3 cell line (Chapter 1).
2. To assess the effect of 4-flourophenylacetamide-acetyl coumarin (which was found effective in curtailing the proliferation) on the mechanism of cell death in the A549 human lung cancer cell line (Chapter 2).
3. To evaluate the possible anti-metastatic and anti-angiogenic property of 4-flourophenylacetamide-acetyl coumarin in the A549 human lung cancer cell line (Chapter 3).
4. To study the effect of 4-flourophenylacetamide-acetyl coumarin on the PI3K/AKT signaling pathway in the A549 human lung cancer cell line (Chapter 4).
5. To evaluate the drug-likeliness, biological reactivity and ligand-target interaction of 4-flourophenylacetamide-acetyl coumarin *in silico* using DFT, molecular docking and a knowledge-based set of the rule (Chapter 5).