Cell cycle
The complex events of cell growth and proliferation, represented as ‘the cell cycle’ has been a target for development of new anti-cancer drugs. Cyclin-dependent kinases or CDKs are the central regulators of the cell cycle. Cancer cells show a deregulated cell cycle either by overexpressing cyclins (which are positive CDK regulators) or by not expressing the naturally occurring CDK inhibitors.

Regulation of cell cycle
The cell cycle consists of four phases, DNA synthesis (S phase), mitosis (M phase) which are separated by the G1 and G2 phases. A number of CDKs (CDK1- CDK9) and cyclins (cyclin A- T) have been identified. Cyclin D isoforms (cyclin D1-D3) interact with CDK2, -4, and -6 and drive the cells through the G1 phase. The coalition of cyclin E with CDK2 helps the cell through the G1/S transition and directs the entry into S phase. Association of cyclin A with CDK2 and cyclin A with CDK1 directs the S and G2 phases, respectively. Cyclin B/CDK1 complex is important for mitosis to happen (Schwartz and Shah, 2005).

The entry of cells from the quiescent phase (G0) to the G1 phase is tightly controlled by a restriction point, R so that the cells can either go to G0 state or proceed to G1. After this transition point (R point), the cells are independent of any external mitogenic stimuli (Pardee, 1974, Schwartz and Shah, 2005). G1 to S phase transition is controlled by the Rb (retinoblastoma tumor suppressor) gene product. The active Rb (hypophosphorylated form) prevents premature cell division by associating with the E2F transcription factors, thereby negatively regulating the G1/S transition. Once inactivated, it allows the cell cycle to proceed. Through G1, growth signals promote the binding of cyclin D to either CDK4 or -6, resulting in Rb hypophosphorylation. Once the cells pass through the R point, Rb is hyperphosphorylated by cyclin E/ CDK2 complexation, with release of bound E2F (Cadoo et al., 2014). This E2F activates S-phase genes and proteins like thymidylate synthase (TS) and dihydrofolate reductase (DHFR) (Schwartz and Shah, 2005). Negative regulators of CDK4/6-cyclin activity include the members of INK4 family viz. p16, p15, p18, p19 (Swanton, 2004, Finn et al., 2009).

In S phase, Cyclin A/CDK2 complex helps in production of proteins involved in DNA synthesis, including histones and proliferating cell nuclear antigen (PCNA). Another checkpoint termed the replication checkpoint at the S phase monitors and slows down the DNA synthesis rate.
During late S and throughout G₂ phases, the cyclin A and B levels are increased. As the level of cyclin B rises, it forms a complex with CDK1 (cdc2) which remains in the cytoplasm. Once the S phase is completed, the cyclin B/CDK1 complex is activated which then relocates to the nucleus and starts mitosis. If the DNA is damaged during the S phase, this activation doesn’t occur and further progression into G₂ and M phases are halted (G₂/M checkpoint). DNA damage activates two kinases, Wee1 and Myt1 which phosphorylate the kinases Chk1/2 which inhibit CDK1. This is competitively regulated by cdc25 phosphatase which removes the above inhibitory signals. Damage to DNA also activates the tumor suppressor p53 protein (Vermeulen et al., 2003, Schwartz and Shah, 2005).

The spindle checkpoint ensures proper chromatid attachment during the metaphase. APC/C (anaphase-promoting complex/cyclosome) protein complex plays notable role in the M phase. The APC-cdc20 complex promotes the entry of cells into anaphase by promoting ubiquitin-mediated degradation of cyclin B and other proteins. When there is a defect in the microtubule, the Mad (mitotic arrest deficient) and Bub (budding uninhibited by benomyl) proteins inhibit the cdc20 subunit of APC-cdc20 which further prevents the entry of cells from metaphase to anaphase (Vermeulen et al., 2003, Fang et al., 1998, Amon, 1999).

Fig. 3.1. Cell cycle and its important regulators
CDK inhibitors and breast cancer

Considering the importance of CDKs in cell cycle, inhibiting them has emerged as a strategy in targeted cancer therapy. Significant section of breast cancer exhibits dysregulated CDK4/cyclin D1/Rb interaction (Mayer, 2015).

One or more cyclins, particularly cyclin D1 were found to be overexpressed in breast cancer samples (Buckley et al., 1993). Estrogen receptors regulate CDK4/6 activity through transcriptional direction of cyclin D1. Cyclin D1 overexpression was found in ER⁺ve (estrogen receptor-positive) breast cancer and reported to be associated with tamoxifen resistance (Rudas et al., 2008, Finn et al., 2009). Palbociclib, abemaciclib, and LEE011 have been developed as selective CDK4/-6 inhibitors against breast cancer (Mayer, 2015). Palbociclib is synergistic with tamoxifen in ER⁺ve and with trastuzumab HER2⁺ve cell lines (Cadoo et al., 2014, Finn et al., 2009) and has been the most promising drug among the ones developed.

Apoptosis

The number of cells in a multicellular organism is tightly regulated by cell division and programmed cell death or apoptosis. The term ‘apoptosis’ was first used by Currie, Wyllie and Kerr to explain the mechanism of cell deletion in a programmed manner (Kerr et al., 1972).

Apoptosis plays a role in normal development, ageing as well as immune responses and a variety of physiological and pathological factors can trigger the process. The apoptotic cells are morphologically characterized by cell shrinkage, dense cytoplasm and pyknosis (nuclear condensation) due to chromatin condensation. This is followed by karyorrhexis (nuclear fragmentation) leading to formation of apoptotic bodies which are then phagocytosed (Elmore, 2007). Since the apoptotic cellular components are packed inside an intact plasma membrane (apoptotic bodies), the constituents are not released into the interstitial space which will prevent any inflammatory responses or necrosis (Savill and Fadok, 2000).

Apoptotic signaling pathways

Apoptosis is an energy-dependent and complex pathway. Many of the current treatment modalities like radiation and chemotherapy works by inducing apoptosis (Makin and Dive, 2001). A family of cysteine proteases, namely the caspases acts as the effector molecules in apoptosis. Apoptosis related caspases include the initiator caspases (-2, -8, -9, and 10) which trigger apoptosis and the executioner caspases (-3, -6 and -7) (Rupinder et al., 2007).
Activation of caspases can be initiated by the death receptor (extrinsic) pathway or the mitochondrial (intrinsic) pathway.

**Extrinsic pathway** involves death receptors which are members of the tumor necrosis factor (TNF) superfamily (Locksley et al., 2001). The finest described ligands and corresponding death receptors comprise TNF-α/TNFR1, Apo2L/DR4, FasL/FasR, Apo3L/DR3, and Apo2L/DR5 (Elmore, 2007). The extrinsic pathway mechanism may be best described using the Fas-ligand (FasL). Activation of the Fas by a death stimulus results in binding of adapter protein FADD and recruitment of FADD which then binds to procaspase-8 through dimerization of the death effector domain. A DISC (death-inducing signaling complex) is formed leading to activation of caspases-8 and further activation of downstream executioner caspases, especially caspase-3. Sometimes, caspases 8 can lead to release of cytochrome c and thereby activation of the intrinsic apoptotic pathway (Ghobrial et al., 2005, Wajant, 2002, Elmore, 2007).

**The intrinsic apoptotic pathway** is non-receptor mediated and a variety of stimuli like toxins, radiation, free radicals etc. can trigger this pathway. Here, the activation of caspases is dependent on the mitochondrial transmembrane potential and permeabilization. The pathway is tightly regulated by the Bcl-2 (B-cell lymphoma-2) family of proteins which is composed of both pro-survival and pro-apoptotic members (Cory and Adams, 2002). The pro-survival group includes Bcl-2, Bcl-XL, Bcl-w, Mcl-1, A1, etc. and the pro-apoptotic group includes Bax, Bak, Bok and the BH-3 group comprising of Bid, Bim, Bad, and others (Um, 2015). A disturbance in the levels of these pro-survival and pro-apoptotic members contribute to apoptosis. Upon disruption of the mitochondrial membrane by the apoptotic stimuli, proteins located in the intermembrane space like cytochrome c, endonuclease G, Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low PI), Omi/HtrA2 (high temperature requirement protein A2), AIF (apoptosis-inducing factor), etc. is released which activates the caspases cascade. Cytochrome c release results in formation of apoptosome (cytochrome c/Apaf-1/caspase-9-containing apoptosome complex) leading to caspases-9 activation. Smac/DIABLO and Omi/HtrA2 neutralize the inhibitor of apoptosis proteins (IAPs) (Saelens et al., 2004, Fulda and Debatin, 2006) and results in apoptosis. Endonuclease G translocates to the nucleus and cleaves the chromatin resulting in DNA fragmentation, which is a characteristic feature of apoptosis (Low, 2003).
The upstream caspases viz., caspase-8 (from the extrinsic pathway) and caspase-9 (from the intrinsic pathway) converge to executioner caspases, -3, -6 and -7 (Ghobrial et al., 2005). Caspase-3 is the major executioner enzyme and it activates the endonuclease Caspase-Activated DNase (CAD) which then degrades chromosomal DNA resulting in chromatin condensation. Caspase-3 also disintegrates the cell into apoptotic bodies (Sakahira et al., 1998, Elmore, 2007).

![Extrinsic and intrinsic pathways of apoptosis](image)

**Fig. 3.2. Extrinsic and intrinsic pathways of apoptosis**

**Epigenetics in breast cancer**

The role of epigenetics in breast cancer has gained attention in recent years. These epigenetic modifications are medicated by two molecular mechanisms (Jaenisch and Bird, 2003):

- DNA methylation
- Histone modification
The relation of DNA methylation to hormonal receptor status and cell proliferation has been studied widely. Gene expression is affected by either hypomethylation or hypermethylation of the DNA.

DNA hypomethylation wherein the methylated genes are demethylated results in expression of normally repressed genes. Eg: HRAS (Harvey Rat Sarcoma Viral Oncogene Homolog). This can lead to cell proliferation and tumorigenesis.

Hypermethylation of the promoter CpG islands leads to suppression or inactivation of hundreds of genes responsible for cell cycle regulation, angiogenesis, apoptosis, tumor suppression, metastasis etc. CpG islands hypermethylation has been also found to result in loss of expression in BRCA1 gene in breast cancer (Esteller et al., 2000).

Silencing of PTEN, which is a negative regulator of PIP3-Akt pathway is also implicated in breast cancer. Loss of expression of this molecule leads to cell survival and decreased apoptosis, due to Akt activation (Muggerud et al., 2010).

DNA is packed as a highly organized complex. Nucleosome, the fundamental chromatin subunits, has four histone proteins around which the DNA is wrapped. The open or closed chromatin states (euchromatin and heterochromatin) are controlled by histones. These histones are exposed to acetylation, deamination, methylation, proline isomerization etc. The DNA which is normally inaccessible due to the compact structure is exposed during gene transcription. The DNA binding proteins can then modify the N-terminal tails of histones. Acetylation of histones are well studied and understood compared to methylation and phosphorylation. Acetylation occurs at the terminal amino groups of the conserved lysine residues. Trimethylation at H3K4, H3K36, or H3K79 leads to a euchromatin which is related with active transcription. Histone acetyltransferases are enzymes which mediate histone acetylation and histone deacetylases (HDACs) remove the acetyl groups leading to repression of transcriptional genes and formation of heterochromatin. In summary, histone acetylation increases transcriptional activity and deacetylation leads to gene repression. A few substrates of HDACs are p53, E2F1, NF-kB, hormonal receptors like AR (androgen receptor), ER (estrogen receptor) and GR (growth receptor), heat shock/chaperone response (HSP90) etc. (Jovanovic et al., 2010).

**Angiogenesis**

The formation of new blood vessels, termed Angiogenesis, is an inevitable step for tumor growth and metastasis. The tumor vasculature provides the route for the cells from primary tumor to escape and reach other body parts (metastasis) (Samant and Shevde, 2011). Broadly,
the activation of endothelial cells (EC) results in the release of proteases which degrades the basement membrane of cells in the existing blood vessel. The cells will then migrate to these new interstitial space and form new vascular tubes. Blood flow is initiated and new vasculature stabilizes (Gacche and Meshram, 2013).

When a tumor exceeds a few millimeters in size, it triggers an “angiogenic switch” to meet the metabolic needs of the cells (Weis and Cheresh, 2011). This switch is regulated by various pro-and anti-angiogenic factors. Vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor, transforming growth factor, platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and angiopoietins are a few pro-angiogenic factors. Some of the anti-angiogenic factors include thrombospondin-1, angiostatin and endostatin (Sakurai and Kudo, 2011).

Tumor cells release various cytokines and growth factors and dysregulate the normal cells around them. For example, VEGF released by the tumor cell increases endothelial cell proliferation and sprouting. The tumor vasculature which is being continuously remodeled being leaky, recruits platelets to the basal lamina. The activated platelets escalate the response by recruiting angiogenic and permeability factors. Tumor associated fibroblasts further deposit extracellular matrix (ECM) proteins. MMPs cleave the ECM to fragments which attracts inflammatory cells to move to the site and release various factors which will modulate angiogenesis (Weis and Cheresh, 2011).

![Fig. 3.3. Steps in angiogenesis and factors influencing the various stages](image)

**Fig. 3.3. Steps in angiogenesis and factors influencing the various stages**  
Ang: angiogenin, Angp: angiopoietin, EGF: epidermal growth factor, FGF: fibroblast growth factor, HIF-1: hypoxia inducible factor-1, HGF: Hepatocyte growth factor, IGF: Insulin-like

**Advantages of angiogenesis-based therapy**
- Chances of drug resistance are less since the endothelial cells are diploid and genetic mutations are highly unlikely.
- Damaging a single vessel will be enough to deprive the tumor cells of nutrition.

**Limitations of angiogenesis based treatments** (Gupta and Zhang, 2005)
- Many pre-clinically successful drugs targeted against angiogenesis failed in clinical trials because the number of proliferating endothelial cells is less in human tumors compared to the rodents. The main target of anti-angiogenic drugs are proliferating endothelial cells.
- Angiogenesis is supported by a large number of factors present in the tumor microenvironment. Hence, targeting a single factor might not be effective.
- Being tumor specific, the tumor microvasculature is highly unpredictable.
- Monitoring the anti-angiogenic effect in a human subject is less feasible.

**Anti-angiogenic drugs** (Samant and Shevde, 2011)
The main approved drugs for targeted anti-angiogenic therapy are the monoclonal antibodies and small molecule tyrosine kinase inhibitors. Apart from these, mTOR inhibitors, proteasome inhibitors and thalidomide are also reported to indirectly inhibit angiogenesis.

**Monoclonal antibodies**
Bevacizumab, cetuximab, Panitumumab and trastuzumab are four FDA approved drugs.
*Avastin (Bevacizumab)* prevents the interaction of VEGF with the VEGF receptors by binding with active forms of VEGF resulting in inhibition of endothelial cell proliferation and angiogenesis. The drug in combination with paclitaxel has been evolved as a first line of treatment in breast cancer patients. It is also an approved drug for the treatment of metastatic colorectal cancer and non-small cell lung cancer.
*Cetuximab and Panitumumab* prevent the binding of ligands to the EGFR (epidermal growth factor receptor) resulting in degradation of receptors and inhibition of angiogenesis and cell proliferation.
*Herceptin (trastuzumab)* which is targeted to block the function of HER2 protein is the first approved humanized antibody for breast cancer treatment.
Erlotinib, sorafenib, sunitinib are approved tyrosine kinase inhibitors for anti-angiogenic therapy. Erlotinib is a selective inhibitor of the EGFR; sorafenib and sunitinib inhibits VEGFR (1, 2 and 3), and PDGFR-β. 

*Rafamycin, deforolimus, everolimus and Temsirolimus* are mTOR inhibitor class of anti-angiogenic agents.

**Metastasis** is the process by which the tumor cells from a primary tumor disseminates and reaches the distant parts in the body. The entire process of “metastatic cascade” involves the following steps (Geiger and Peeper, 2009):

- Tumor cell dissemination from primary tumor and their epithelial to mesenchymal transition (EMT) of the cells to acquire invasive properties.
- Degradation of basement membranes and remodeling of the extracellular matrix (ECM) by proteinases to facilitate invasion.
- Invasion and migration of the cells.
- Angiogenesis and intravasation of tumor cells into newly formed vessels within or nearby the tumor.
- Extravasation of tumor cells from the blood vessels.
- Disseminated cells grow out to a secondary tumor / macrometastasis.

**Models to explain metastasis**

Over the decades various models have been proposed to explain metastasis (Hunter et al., 2008).

**a) Progression model**

The model proposed by Nowell suggests that a primary tumor gains full metastatic potential through a series of somatic mutations in the subpopulation (Nowell, 1976).

**b) Fusion model**

Metastatic cells acquire lymphoid characteristics either by fusion with myeloid cells or by uptake of DNA tumor present in circulation.

**c) Transient compartment model**

At a given period of time only a few viable cells are able to complete metastasis due to random epigenetic events. Hence not all cells within a tumor will retain the capacity to form colonies at secondary sites.

**d) Gene transfer model**
The tumor DNA (carrying mutations) present in the circulation is carried to the secondary site where they will be absorbed by the stem cells. This awards the stem cells with malignant properties.

e) Early oncogenesis model
The metastatic potential of a tumor is established early in oncogenesis as a result of somatic mutation. This is due to the same events of activation and inactivation resulting in the primary tumor.

f) Genetic predisposition model
The genomic diversity affects a tumor’s metastatic progression. The inherent polymorphism will influence the metastatic potential by affecting the expression of pro-metastatic genes.

Matrix metalloproteinases (MMPs)
Tumor angiogenesis, invasion and metastasis require degradation of the extracellular matrix (ECM). The MMPs are zinc-containing endopeptidases capable of degrading various components of the ECM (Rundhaug, 2003). The most common and studied MMPs are the collagenases (MMP-1,-8,-13), gelatinases (MMP-2,-9) and stromelysins (MMP-3,-10,-12), named based on the specificity of the components they degrade (Westermarck and Kahari, 1999). MMPs help to provide a favorable microenvironment for the tumor cells by remodeling the ECM as well as by releasing the growth factors (Rundhaug, 2003). MMP-2 and -9 can be easily detected in body fluids and has been studied as a possible prognostic marker for breast cancer (Vasaturo et al., 2013). MMP-2,-9 and -14 are believed to be key players in tumor angiogenesis. MMP-9 increases the availability of key growth factors like VEGF and FGF, and thereby plays an important role in angiogenic switch (Sakurai and Kudo, 2011). Many MMPs (MMP-1, -2, -3, -7, -9, -11, -19) release the precursors of growth factors present in cell membrane which promote cell proliferation, such as those of IGFs and EGFR. MMP-2,-3,-9,-13 and -14 has been found to be overexpressed during EMT (Gialeli et al., 2011).

Matrix metalloproteinase inhibitors (MMPIs) (Gialeli et al., 2011)
Some potential MMPIs developed are:

- Peptidomimetic MMPIs: having structural similarity with collagen, they function as competitive inhibitors at the MMP-9 cleavage site. Eg: Batimastat, Marimastat
- Nonpeptidomimetic agents: these agents were developed based on the 3D conformation of the active site of MMP. Eg: Tanomastat, Prinomastat, BMS-275291
Chemically modified tetracyclines: they inhibit both the synthesis and enzymatic activity of MMPs. Eg: Metastat, Minocycline

Off-target inhibitors: few drugs show MMP inhibition apart from their primary target. Eg: Bisphosphonates, Letrozole. Bisphosphonates inhibit the MMPs besides their anti-osteoclast activity. Letrozole, which is a nonsteroidal P450 aromatase inhibitor, also inhibits MMPs.

Natural inhibitors: Genistein (soy isoflavone), Neovastat (from shark cartilage)

Hypoxia-inducible factor 1 (HIF-1) in cancer
HIF-1α has emerged as a vital transcription factor in breast and prostate cancer biology, and its expression is correlated with diagnostic and prognostic indicators for relapse and metastasis.

Hypoxia is a common feature of the tumor microenvironment and hypoxia-inducible factor 1 (HIF-1) plays an important role in the tissue’s adaptive response to this. As cells proliferate, the oxygen demand also increases which will result in hypoxia leading to activation of HIFs. This indeed results in transcription of VEGF (vascular endothelial growth factor) gene, stimulates angiogenesis and increases oxygen supply (Semenza, 2012).

HIF-1 is a transcriptional factor consisting of α and β-subunits. HIF-1α is oxygen sensitive and HIF-1β is oxygen-insensitive (Wouters et al., 2004). Both the subunits have a basic helix-loop-helix-PAS (bHLH-PAS) domain for DNA binding and heterodimerization (Wang et al., 1995). Under normoxic conditions, HIF-1α is regulated by ubiquitination which is mainly mediated by the von Hippel-Lindau (VHL) protein. Prolyl hydroxylase, PHD2 mediate the hydroxylation of proline residues in HIF-1α resulting in interaction with VHL which is a member of the the E3 ubiquitination complex (Semenza, 2012, Jaakkola et al., 2001). Finally, HIF-1α undergoes proteasomal degradation. PHD2 activity requires oxygen for hydroxylation and hence hypoxia prevents the ubiquitination of HIF-1α (Epstein et al., 2001).

Hence the HIF-1α gets stabilized and enters the nucleus where it associates with HIF-1β and activates the transcription of many genes (Wouters et al., 2004).

HIF-1 activation leads to upregulation of many genes involved in angiogenesis, metastasis, invasion, glucose metabolism, survival etc. (Hong et al., 2004).
Table 3.1. Genes upregulated by HIF-1

<table>
<thead>
<tr>
<th>Angiogenesis</th>
<th>Survival and Proliferation</th>
<th>Glucose metabolism</th>
<th>Invasion and Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENG</td>
<td>Cyclin G2</td>
<td>HK1</td>
<td>KRT14</td>
</tr>
<tr>
<td>LEP</td>
<td>TGF-α</td>
<td>HK2</td>
<td>KRT18</td>
</tr>
<tr>
<td>LRP1</td>
<td>TGF-β3</td>
<td>GLUT1</td>
<td>KRT19</td>
</tr>
<tr>
<td>TGF-β3</td>
<td>ADM</td>
<td>GLUT3</td>
<td>VIM</td>
</tr>
<tr>
<td>VEGF</td>
<td>EPO</td>
<td>GAPDH</td>
<td>Collagen type V</td>
</tr>
<tr>
<td>VEGFR</td>
<td>NOS2</td>
<td>LDHA</td>
<td>FN1</td>
</tr>
<tr>
<td>ADM</td>
<td>IGF2</td>
<td>PKM</td>
<td>MMP2</td>
</tr>
<tr>
<td>ET1</td>
<td>VEGF</td>
<td>TPI</td>
<td>Prolyl-4-hydroxylase α</td>
</tr>
<tr>
<td>NOS2</td>
<td>Transferrin</td>
<td>ALDA</td>
<td>UPAR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ALDC</td>
<td>LRP1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LEP</td>
<td>TGF-α</td>
</tr>
</tbody>
</table>


**Anti-cancer agents that inhibit HIF-1 activity**

Numerous drugs have been reported to inhibit HIF-1 through a variety of mechanisms and many are in clinical use (Semenza, 2012, Semenza, 2007). A few anti-cancer drugs with their mechanism of action and targets are presented in Table 3.2.
### Table 3.2. Anti-cancer drugs with anti-HIF-1 activity

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Drugs and targets</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased HIF-1α mRNA levels</td>
<td>Aminoflavone</td>
<td>(Terzuoli et al., 2010)</td>
</tr>
<tr>
<td></td>
<td>Temsirolimus (mTOR)</td>
<td>(Majumder et al., 2004, Shackelford et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Topotecan (Topoisomerase I)</td>
<td>(Kummar et al., 2011)</td>
</tr>
<tr>
<td>Decreased HIF-1α protein synthesis</td>
<td>NSC64422 (Topoisomerase II)</td>
<td>(Creighton-Gutteridge et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>Flavopiridol (cyclin-dependent kinases)</td>
<td>(Newcomb et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>2-methoxyestradiol (microtubules)</td>
<td>(Escuin et al., 2005)</td>
</tr>
<tr>
<td>HIF-1α protein stabilization</td>
<td>17-allylamino-17-demethoxygeldanamycin, Apigenin (HSP90)</td>
<td>(Liu et al., 2007) (Osada et al., 2004)</td>
</tr>
<tr>
<td></td>
<td>LAQ824 (Histone deacetylase)</td>
<td>(Qian et al., 2006)</td>
</tr>
<tr>
<td>Block HIF-1α:HIF-1β dimerization</td>
<td>Acriflavine</td>
<td>(Lee et al., 2009b)</td>
</tr>
<tr>
<td>Inhibit HIF-1 DNA binding</td>
<td>Echinomycin</td>
<td>(Kong et al., 2005)</td>
</tr>
<tr>
<td></td>
<td>Doxorubicin, daunorubicin</td>
<td>(Lee et al., 2009a)</td>
</tr>
<tr>
<td>Decreased HIF-1 mediated transactivation</td>
<td>Bortezomib (Proteasomes)</td>
<td>(Kaluz et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Vorinostat (Histone deacetylase)</td>
<td>(Fath et al., 2006)</td>
</tr>
</tbody>
</table>

### ROS, inflammation and cancer

Oxidative stress arises when there is an imbalance between the cell’s anti-oxidant defense mechanisms and the production of ROS (reactive oxygen species). This has been implicated to have a substantial role in various conditions like cancer, inflammation, ageing and neurodegenerative diseases. Studies have reported that cancer cells are under more oxidative stress than the normal cells. This arises due to high mitochondrial/metabolic activity, oncogenic signaling, cellular crosstalk with immune cells etc. (Liou and Storz, 2010). ROS
can induce genetic lesions through DNA damage which can lead to tumorigenicity and its progression. They can also help in cancer cells’ survival and migration (Storz, 2005).

The association between cancer and inflammation has long-been studied. The chronic inflammatory environment is dominated mainly by the macrophages which along with leukocytes produce reactive oxygen and nitrogen species. Peroxynitrite is formed due to the reaction of these species, which is a mutagenic agent (Coussens and Werb, 2002). DNA damage may be aggravated by migration inhibitory factor (MIF) and tumor necrosis factor-α (TNF-α) produced by the macrophages and T lymphocytes. MIF also favors mutations by impairing the p53 tumor suppressor responses (Lu et al., 2006, Hudson et al., 1999)

**Fig. 3.4. Association between inflammation and cancer.** Bax: Bcl2-associated X protein, Bcl-2: B-cell lymphoma 2, COX2: cyclooxygenase2, ICAM-1: intercellular adhesion molecule 1, IL-1, -6: interleukin-1, interleukin-6, MMP-9: matrixmetalloproteinases-9, NFkB: nuclear factor kappa B, TNF- tumor necrosis factor, VEGF: vascular endothelial growth factor.

In inflammation-associated cancer, NF-κB protects the transformed tumor cells from elimination by endogenous apoptotic factors. The downstream molecules like iNOS (inducible Nitric Oxide Synthase), COX-2, HIF-1α, IL-6, TNF-α, MMPs and adhesion
molecules are also regulated by NF-κB, which makes it an important molecule linking cancer and inflammation (Li and Verma, 2002, Lu et al., 2006).

It has been reported that breast cancer metastasis is governed by chemokine receptors. Human breast cancer cells have shown a high expression of CXCR4 and CCR7 chemokine receptors. These receptors induced the chemotactic and invasive responses in breast cancer cells through polymerization of actin and pseudopodia formation. CXCR4 is also expressed in prostate cancer, chronic lymphocytic leukemias, B-cell lymphomas etc. which might imply that chemokines may regulate metastasis of various cancer types (Muller et al., 2001, Coussens and Werb, 2002).

**In vivo models in cancer research (Workman et al., 2010)**

Preclinical animal models have been used for years in cancer research to assess the efficacy and safety of anti-cancer agents. Selection of a suitable animal model should be based on its relevance to human cancers in terms of organ site specificity and pathology. The model should be able to produce consistent tumor within a less period of time.

The in vivo cancer models maybe broadly divided into two:

- Transplantation tumor models and
- Autochthonous tumor models, where the tumors arise or are induced in the same host.

**Transplantation models** involve maybe either syngeneic (tumor cells from mouse or rat are transplanted into a same host species) or xenogeneic (human tumor cells are injected into immunodeficient mice).

- **Syngeneic animal models** cover a wide range of tumor types and they models immune and stromal interactions. Few examples of the models are MC26 colon cancer in BALB/c mice, B16 melanoma in C57/Bl mice and 4T1 mammary carcinoma in BALB/c mice. The disadvantages of these models are that they cannot be used if any human specific parameters are being studied and the genetics of the tumors may not be reflecting the human situation.

- **Xenogeneic animal models** have the advantage that they allow direct administration of human cancer cells or primary tissue, subcutaneously or orthotopically. Few examples of these models are HCT116 colon cancer in athymic mice, PC3 prostate cancer in athymic mice and systemic leukemia in irradiated NOD/SCID mice. The
disadvantages of these models are that they require immunodeficient animals and these models may not be suitable where agents affecting immune system are studied.

**Autochthonous tumor models** can be either primary tumor models or models using genetically engineered mouse.

**Primary tumor models** maybe:

- **Chemically-induced:** These models have the advantage that they models the entire carcinogenic events. Examples of these models are Dimethyl hydrazine induced gastric cancer, Azoxymethane induced colon cancer, DiethylNitrosamine induced heptaocellular carcinoma, Dimethylbenz(a)anthracene (DMBA) induced breast cancer and Dimethylbenz(a)anthracene/ tetradecanoyl phorbol acetate (TPA) induced skin cancer. Disadvantages with these models are that the incidence rates are low and tumor development is time consuming.

- **Inflammation-induced:** These models are very limited in number, but involve full spectrum of immune mediators. Example of such a model is Helicobacter pylori-induced gastric cancer in gerbils.

- **Surgically-induced:** Example of this model is Oesophago-gastroduodenal anastomosis model of oesophageal carcinogenesis. This model can present metastatic spread.

**Genetically engineered mouse models** include mammary carcinomas induced by the viral oncogene polyoma virus middle T or by Her2/neu oncogene, colon adenomas and carcinomas induced by inactivation of the adenomatous polyposis coli (APC) tumour-suppresser gene, retinoblastoma by Rb gene, PTEN model for breast, prostate, endometrial and thyroid carcinoma, Nf1 model for pheochromocytoma etc.

**Nutraceuticals in cancer and inflammation**

A large number of natural products showed great potential for targeting cancer at various phases. Many of them are having multiple molecular targets and acts at various stages of cancer like cell cycle, apoptosis, angiogenesis, and invasion as well as in inflammation. They can inhibit or prevent carcinogenesis by virtue of their ability to induce various antioxidant enzyme systems too. Data from epidemiological studies have also revealed that dietary intakes of fruits and vegetables reduced cancer risk (Pratheeshkumar et al., 2012).

Few targets and their inhibitors from natural sources are (Aggarwal et al., 2009):
Bcl-2- Allyl isothiocyanate, β-carotene, β-sitosterol, capsaicin, cinnamaldehyde, curcumin, gingerol, limonene, lutein.

Bcl-xL- β-carotene, capsaicin, allyl isothiocyanate

Bax- Cinnamaldehyde, curcumin, limonene, lutein, diallyl sulfide

Survivin- Capsaicin, curcumin

Caspases- Allicin, β-sitosterol, cinnamaldehyde, citral, kaempferol, limonene, myristicin, shogaol

p-53- Lutein, limonene, shogaol, diallyl sulfide, quercetin

Cyclin D1- Apigenin, capsaicin, curcumin, lutein, sesamin, sulforaphane

MMP-9- Caffeic acid, curcumin, ursolic acid, vanillin, quercetin

ICAM-1- Allicin, Apigenin, crocetin, kaempferol

VEGF- Caffeic acid, capsaicin, curcumin, diallyl disulfide, gingerol, sulforaphane

NFκB- Cinnamaldehyde, quercetin, curcumin, sulforaphane.

TNF- Ajoene, curcumin, eugenol, curcumin, gingerol, kaempferol

IL-6- Diallyl sulfide, piperine, phylic acid

IL-8- Phylic acid, allicin

Glycosmis pentaphylla (Retz.) DC.

Family: Rutaceae

Synonyms: Glycosmis arborea (Roxb.) DC., Glycosmis pentaphylla (Retz.) Correa.

Vernacular names: Ban Nimbu (hindi), kattukonchi (kannada), Asvasakhotah (Sanskrit) kuttipanal, panchi (malayalam).
This aromatic shrub is distributed throughout India especially as undergrowth in forests. The plant is a bitter, astringent, febrifuge, expectorant and anti-inflammatory. The leaves has been used in hepatopathy, rheumatism, jaundice, anaemia and wounds (Warrier et al., 2004, Satyavati et al., 1976). Roots are used as febrifuge and wood is used in snake bite (anti-venom). The plant is an anti-cancer medicine (Panda, 2002). The traditional healers in Gazipur district of Bangladesh use the plant to treat all forms of cancers (Mollik et al., 2010, Sreejith et al., 2012b).

- The plant has been a source of various alkaloids of acridine and carbazole classes. Compounds like acridones, furoquinalones, carbazole alkaloids, triterpenoids and sulfur containing amides have been reported in the plant (Quader et al., 1999).
- Carbazole alkaloids- glycozolidal (Bhattacharyya and Chowdhury, 1985b) and glycoborinine (Chakravarty et al., 1999) from roots, methyl carbazoles (Bhattacharyya et al., 1987), glycolone (a quinolone alkaloid) from leaves (Bhattacharyya and Chowdhury, 1985a), arborinine (an acridine alkaloid) (Banerjee et al., 1961), hydroquinone diglycoside acyl esters from stem (Wang et al., 2006a), skimmianine, glycosminine, glycosine (Chatterjee and Majumdar, 1954) has been reported.
- Arborinine inhibited the growth of crown gall tumors produced by Agrobacterium tumefaciens in a potato disc bioassay (Quader et al., 1999).
A hydroperoxyquinolone alkaloid, glycopentaphyllone (Sripisut et al., 2012) from fruits has been isolated.

Phenolic glycosides (glycopentosides A-C) (Tian et al., 2014), carbazole phytoalexins (Pacher et al., 2001) few isoflavone diglycosides (Wang et al., 2006b), flavanols, glycoflavanones A and B (flavonols), betasitosterol, alphaltol, oxyresveratrol (Wu et al., 2012) and four bioactive flavonoids (Khan et al., 2013) were isolated from the plant.

Glycoborinine (carbazole alkaloid) has been studied for its potential against HepG2 cells (Yang et al., 2014).

The carbazole alkaloids, Glycosmisines A and B, isolated from the stems of the plant showed cytotoxicity against A549, HepG-2 and Huh-7 cells (Chen et al., 2015) and glybomines A, B and C exhibited inhibitory effects on Epstein–Barr virus early antigen (EBV-EA) induction (Ito et al., 2004).

Crude extracts and fractions of the whole plant and an isolate, glycopentalone have shown anti-hepatocellular carcinoma activity (Sreejith et al., 2012a, Sreejith and Asha, 2015).

Anti-arhritic activity and anti-diabetic activities of the plant in a polyherbal formulation (Petchi et al., 2015, Petchi et al., 2014), anti-microbial activities of isolated alkaloids (Chen et al., 2012), wound healing activity of the leaf extract (Silambujanaki et al., 2011) and hepatoprotection of plant (Gomes et al., 2003, Nayak et al., 2011, Mitra and Sur, 1997) has been reported.

The plant has not been extensively studied for its effect on breast cancer or on any specific cancer models in vivo.
References


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