Introduction
"Few diseases have the power of inspiring fear to the same degree as cancer" addressed Prof. W. Wernstedt in nobel prize award ceremony (1926). Cancer is still the most progressive and dreadful disease in spite of significant advancements in medical technology for its identification and treatment.

**Cancer**

Cancer is a disease in which there is attenuation of control of cell proliferation and death in the tissues affected. It is the common term used to denote malignant neoplasms in humans. It is characterised by a) rapid creation of abnormal cells with reduced or unrestricted control of growth b) invasion to adjoining tissues and c) spread or metastasis to other parts of the body.

**Cancer incidence**

"Each minute, in a body a considerable number of cells undergo cell death. When a new cell replaces a dead cell, homeostasis is maintained. The balance between proliferation and death of a cellular population is altered when death events occur to an extent lower than normal, thus leading to cell accumulation disorders".

As indicated by the most recent World Health Organisation (WHO) statistics, cancer is the second heading reason of mortality globally. WHO reports 14.1 million new cancer cases and 8.2 million cancer deaths (7.9 million every year). About 32.6 million individuals were living with this malignancy (within 5 years of diagnosis) in 2012 around the world. 60% of world's aggregate new annual cases are reported in Africa, Asia and focal and south America [1-3]. On an average, 70% of cancer patients in developing countries are diagnosed at the end stage of ailment. Cancer in the developing countries is predicted to increase to 6.7 million by 2015 and 8.9 million by 2030 [4].

“A disease once associated with affluence now places its heaviest burden on poor and disadvantaged populations” said Margaret Chan, present WHO Director-General.
Cancer affects any organs in people of all ages. Apart from the individual sufferer, the economic burden to society is immense.

**Breast cancer and its incidence**

Breast cancer is considered to be one of the most common cancer threats worldwide. The mammary gland consists of epithelial cells at various differentiating phases. Basal and luminal epithelium denote two cell populations that arise from a common progenitor. However they express unique markers and perform unique functions individually [5-7]. The St. Gallen International Expert Consensus suggested a classification system of invasive breast carcinomas centred on the expression of: estrogen receptor, progesterone receptor, HER2 and Ki-67 [5], into five different molecular subtypes; luminal A, luminal B (HER2−), luminal B (HER2+), HER2, and basal-like subtypes [8]. 70% of breast cancers are estrogen-dependent luminal epithelial type [9] and about 15% of breast cancers display some characteristics of basal epithelium. MCF-7 cells and ZR-75-1 cell lines are widely used as models of luminal breast cancers [10, 11].

**Fig. 1. Molecular classification of breast cancer [10-12]**

**Incidence:** According to the WHO, breast cancer is the second most fatal cancer in women, with more than 5,19,000 deaths attributed to it globally each year. Breast cancer comprises 23% of all cancers afflicting women globally [13, 14]. The occurrence of breast cancer in women is escalating in developing countries such as India. As per Indian Council of Medical Research (ICMR), frequency of breast cancer in India is on the ascent and it is rapidly becoming the leading cancer in women pushing the cervical cancer to 2\textsuperscript{nd} place [15]. It is accounted that one in 22 women in India is liable to struggle with breast cancer amidst her lifetime [16, 17]. Currently, India reports roughly 100000 new cases of breast cancer, annually. A 2005 survey lead by the International Association of Cancer
Research, based in Lyon, France, estimated that there would be 250000 cases of breast cancer in India by 2015, with an increase of 3% per year [18].

**Head and neck cancer and its incidence**

Squamous cell carcinoma (SCC) is the most recurrent malignant tumour of head and neck region [19]. Head and Neck Squamous cell carcinoma (HNSCC) is a heterogeneous class of malignancy originating in the squamous cells that make up the epithelium in the head and neck region, including the oral cavity, pharynx and larynx [20]. Laryngeal squamous cell carcinoma is one of the most common HNSCC. 95% of laryngeal cancers are SCCs [21]. It is an aggressive malignancy with higher rate of occurrence in men. HNSCC represents 6% of all cancers and is the 6th leading cancer by prevalence globally. Every year nearly 600,000 new cases are diagnosed with HNSCC with 300,000 deaths [22-23]. HNSCC is prevalent in countries with high alcohol and tobacco consumption like Africa, Australia, Brazil, France, India, Netherlands and Switzerland [24]. India has the highest rate of HNC and it accounts for 20% of all cancer types in India [25].

**Etiology of cancer**

Carcinogenesis is a multistage process that can be triggered by external factors like various environmental agents (cigarette smoke, industrial emissions, gasoline vapors), chemical agents (phorbol esters and okadaic acid), physical agents (radiation) and biological agents (tobacco, infectious organisms, viruses etc.) called carcinogens and internal factors such as inherited mutations, hormones, inflammatory agents (tumour necrosis factor (TNF) and $\text{H}_2\text{O}_2$), and mutations that occur from metabolism. These causal factors may act together or in sequence and damage or alter the DNA, transform genes controlling cell proliferation, differentiation and apoptosis thereby initiating or promoting carcinogenesis. Carcinogenesis results in modulated molecular events which involve protein kinases (IKK, EGFR, HER2, JNK, MAPK), anti-apoptotic proteins (Akt, Bcl-2), cell cycle proteins (cylclins, cyclin-dependent kinases), transcription factors (NF-κB, AP-1, STAT5, STAT3), cell adhesion molecules, COX-2, and growth factor signalling pathways [26].
Risk of breast cancer includes but not limited to age, socioeconomic status, family history, life style, diet, alcohol, body mass index, height and exposure to radiation [27]. Major determinants of HNSCC are tobacco smoking, alcohol drinking, unhealthy diet [28] and occupational exposures to polycyclic aromatic hydrocarbons, metal dust, cement dust, varnish, lacquer, etc. Substantial relations were observed with diesel exhausts, ionizing radiation, sulphuric acid mists, and mustard gas [29].

**Cellular and molecular mechanisms of carcinogenesis**

Carcinogenesis involves deregulation of numerous biochemical pathways and molecules (Fig. 2). These comprise the transcription factors, growth factors, growth factor receptors, enzymes, cytokines and genes regulating proliferation and apoptosis.

**Fig. 2. Schematic illustration of multistep carcinogenesis [30]**

It consists of 3 separate but interconnected processes: Tumour initiation, promotion and progression (Fig. 3, 4).

i) Tumour initiation: Tumour initiation is defined as irreversible modification of the cellular DNA. It is achieved by accumulation of genetic changes in a single cell [31] which occurs after exposure to carcinogens/mutagens. Thus initiation corresponds to the introduction of mutation and during this phase the mutant cells proliferate very slowly. However in initiation process, there will not be any changes in the morphology of cellular tissue but it convokes an increase in susceptibility to cancer formation [32].
ii) Tumour promotion: Tumour promotion denotes the growth of initially damaged or mutated cells by inflammation or wounding. This results in the formation of a clone of actively proliferating multicellular premalignant/benign tumour cell population, which may regress without further stimuli [33]. Enhanced cellular proliferation and localized increase in vascular density and blood flow were observed in this phase. Tumour promoters may cause upregulation of some of the receptor signalling pathways like epidermal growth factor receptor (EGFR) [34]. In contrast to initiators, tumour promoting agents usually cause dramatic morphological and biochemical effects that are reversible in the absence of treatment. In this phase, the initiated cell expands clonally to give rise to a population of initiated cells. Clonal selection of partly transformed cells can extensively increase the cell population with acquired mutations critical for carcinogenesis thereby increasing the probability of a subset of these cells to acquire the remaining mutations required for malignant transformation. There is evidence that clonal expansion of premalignant cells is a cardinal feature of carcinogenesis in many tissue and organ sites [35].

iii) Tumour progression and malignant transformation: Malignant neoplasms have copious phenotypic characteristics like disproportionate growth, invasiveness and metastases. These features are acquired in a stepwise manner during tumour progression. The genetic changes that fuel tumour progression involve not only growth regulatory genes but also genes that regulate angiogenesis, invasion and metastases. The process of progression of neoplasm to malignant cells is termed as metastatic cascade. The metastatic cascade is subdivided into 2 phases A) Invasion of extra cellular matrix (ECM), where detachment of tumour cells from each other, attachment of tumour cells to matrix component, degradation of ECM and migration of tumour cells through the basement membrane occur. In this phase, cells acquire the ability to secrete proteases such as matrix metalloproteinases (MMP) and kallikrein that dissolve barriers such as basement membranes, to alter the proteins like of E-cadherin, β-catenin, integrin to tether them to their surroundings and to undergo angiogenesis [36]. B) Vascular dissemination and homing of tumour cells.
Fig. 3. Pathways of carcinogenesis [37]

Fig. 4. Molecular pathways of carcinogenesis [38]
The hallmarks of cancer

The hallmarks of cancer comprise seven biological abilities acquired by the transforming cells during carcinogenesis (Fig. 5). The hallmarks set up an organizing principle for streamlining the complexities of neoplastic disease. They include

1) Self-sufficiency in growth signals
2) Insensitivity to anti-growth signals
3) Evading apoptosis
4) Limitless replicative potential
5) Sustained angiogenesis
6) Tissue invasion & metastasis
7) Inflammatory microenvironment

Fig. 5. Hallmarks of cancer [39]
1. Self-sufficiency in growth signals

Tumour cells generate their own internal growth signals that are not dependent on the surrounding environment. Genes that stimulate autonomous cell growth in cancer cells are termed as oncogenes. Their counterparts in normal cells are known as proto-oncogenes. Oncogenes mimic normal cellular growth signalling pathways but their products, oncoproteins are devoid of essential regulatory elements and are produced in cancer cells without any stimuli. Strategies utilized by cancer cells to gain self-sufficiency in growth signals can be grouped on the basis of their role in the signal transduction cascade and cell cycle regulation. Some of the changes acquired to attain self-sufficiency in growth signals are as follows

(i) Growth factor receptors

Many oncogenes that encode growth factor receptors are known. Mutation and pathologic overexpression of normal forms of growth factor receptors are detected in several tumours. Mutant receptor proteins give continuous mitogenic signals to cells even in the absence of growth factor. Overexpression of growth factor receptors is more common than mutations. It makes cancer cells hyper responsive to normal levels of growth factors. The best documented examples of overexpression involve the EGFR family.

EGFR

The EGFR (EGFR; ErbB-1; HER1) is the cell-surface receptor of the epidermal growth factor family (EGF-family). It is one of the members of HER/erbB family of receptor tyrosine kinases [40-42] which includes HER1 (EGFR /erbB1), HER2 (neu, erbB2), HER3 (erbB3), and HER4 (erbB4) [41, 43]. The structure of EGFR glycoprotein includes an extracellular ligand binding domain, a hydrophobic transmembrane region, a cytoplasmic domain containing the tyrosine kinase domain [44]. Binding of ligand to EGFR initiates receptor dimerization, autophosphorylation of cytoplasmic domain tyrosyl residues, and subsequently Src-mediated activation of the downstream targets and pathways leading to modulation of cellular key functions including proliferative activity and apoptosis [45-48]. Generally, EGFR is present only at low levels in many normal tissues [49]. EGFR overexpression or mutation is frequently related with advanced tumour
stages and metastasis and is reported in a variety of human tumours. EGF-related growth factors act through autocrine and paracrine mechanisms [50]. Aberrantly enhanced EGFR signalling is connected with breast cancer, head and neck cancer, renal cancer, ovarian cancer etc [40]. The frequency of overexpression of EGFR in human head and neck carcinomas is 100% [51, 52]. Its overexpression is considered as a major predictive factor in head and neck cancer patients [53], particularly in HNSCC [54-57].

(ii) Signal transducing proteins

Cancer cells also gain growth autonomy by mutations or over expression of genes that encode signal transducing proteins. These signalling molecules couple growth factor receptors to their nuclear targets. Many such signalling proteins are connected with the inner leaflet of plasma membrane. They receive signals from activated growth factor receptors and help to transmit these signals to the nucleus. Some signal transducers that contribute self-sufficient growth to cancer cells are:

Src

The link between src and EGFR is well-known [58]. Src, a non-receptor tyrosine kinase protein is involved in cell differentiation. It functions in regulating normal cellular growth. It imparts malignant properties to cancer cells through aberrant regulation of growth signals. Src, tethered to cell membranes, is essential to control the actin and adhesion network inside cells. Src also plays a vital role in life or death decisions taken by cells. In cancer cells, many of the above said events are impaired. Therefore recognizing the role played by deregulated src in tumour progression will be of intense value for cancer treatment. The src pathway has been observed to be activated in about 50% of tumours from colon, liver, lung and the pancreas [59]. It is also activated in MCF-7 cells [60]. Elevated expression levels of src were found in human breast cancer tissues [61, 62].

Activated src promotes survival (PI3K, Akt, NF-κB), angiogenesis (STAT3, VEGF, IL-8), proliferation (Ras, Raf), and invasion (c-Jun) processes. It is known that src forms a complex with STAT3 and that src-mediated phosphorylation activates STAT3 [63-66].
**Phosphoinositide 3-Kinase (PI3K): A multifunctional signalling molecule**

The PI3K are ubiquitous, heterodimeric lipid and serine/threonine kinases. They are activated by diverse extracellular signals. They are classified into 3 major subfamilies based on their structure and the substrate specificity as Class I, Class II and Class III PI3Ks [67]. PI3K activates Akt for regulating cellular functions. It orchestrates cell responses such as mitogenic signalling, cell survival and growth, proliferation, metabolic control, vesicular trafficking, degranulation, cytoskeletal rearrangement and migration. The PI3K/Akt pathway plays a main role in regulation of cell cycle progression and proliferation [68, 69].

**Akt**

Akt is otherwise known as protein kinase B is a member of the family of PI3K regulated Ser/Thr kinases [70]. It is the major downstream target of activated PI3K [68]. It is also activated by growth factors like VEGF, FGF, EGF, HGF, IGF [71-74], oncogenes like ras and src, mutated PTEN [75-77]. Activated Akt phosphorylates downstream targets such as Bad [78], p53, caspase-9 [79], glycogen synthase kinase (GSK)-3β [80] and XIAP [81]. Akt thus controls cell survival, cell cycle progression, migration, proliferation, metabolism, tumour growth, and angiogenesis [82, 83]. The PI3K/Akt pathway activation is common in many types of cancer.

(iii) **Nuclear transcription factors**

Ultimately, all signal transduction pathways reach the nucleus through transcription factors and impacts a set of responder genes that coordinate the cells methodical progress through the mitotic cycle. Growth autonomy in cancer cells may happen due to mutated genes regulating the DNA transcription. Many oncoproteins like myc, AP-1 (Jun/Fos), Rel are localized to the nucleus.
**Nuclear factor kappa B (NF-κB) - A ubiquitous transcription factor**

NF-κB is named for its ability to act as an enhancer element of the immunoglobulin kappa light chain gene in B-cells. Mammalian Rel/NF-κB transcription factor family consists of 5 members and grouped into two classes: the Rel proteins - RelA (p65), RelB and c-Rel and the NF-κB family - NF-κB1 (p105/p50) and NF-κB2 (p100/p52). They function as hetero or homodimeric transcription factors. They are known to be activated by 450 different activators (Fig. 6) through two pathways: classical or canonical pathway (proinflammatory cytokines including TNF-α, IL-1, bacterial LPS, and growth factors that act through EGFR, other growth factor receptors and non-receptor tyrosine kinases); an alternative or non-canonical pathway (TNF family) [84, 85]. In resting cells, NF-κB is present in inactive form. It is sequestered in cytoplasm as a heterotrimer complex. This complex comprises of p50, p65 and inhibitory subunit IκB. In response to a range of inducers, IκB kinase (IKK) is activated, which phosphorylates IκB. Phosphorylated IκB undergoes polyubiquitination and consequent proteolytic degradation, releasing NF-κB. In the inactive state, p65 is retained in the cytoplasm. p65 is activated mainly by phosphorylation and acetylation by several protein kinases including PKA, PKCδ and casein kinase II which directly phosphorylate p65. However PI3K/AKT and NF-κB inducing kinases phosphorylate IKK, sequentially phosphorylating p65. After phosphorylation, p65 containing the transactivation domain moves from cytoplasm to nucleus. Inside, it binds to cis-acting κB element and transcripts more than 500 genes [86], many of which encode cytokines/chemokines and their modulators, immunoreceptors, cell adhesion molecules, acute phase proteins, stress response genes, cell surface receptors, regulators of apoptosis, growth factors, ligands and their modulators, early response genes, transcription factors and regulators, enzymes, etc. Thus, NF-κB plays important roles in the control of cell survival, cell proliferation, oncogenesis, invasion, angiogenesis, cell transformation and mediating inflammation [87]. Abnormal constitutive activation of NF-κB /Rel is reported in tumours as well as in cultured cancer cells [88].
STAT (Signal transducers and activators of transcription)

STAT proteins are named due to their twin role as signal transducers and transcription activators. They are dormant cytoplasmic transcription factors and require phosphorylation for nuclear retention. Mammalian STAT family includes 7 members which include STAT1, STAT2, STAT3, STAT4, STAT5 (A&B) and STAT6 [90]. Activation of receptors with intrinsic tyrosine kinase activity (EGFR, PDGFR etc.), intracellular Janus kinase, and various other tyrosine kinases phosphorylates STAT protein and promotes their dimerization through SH2 domain. These STAT dimers translocate to nucleus and bind with their target gene promoters to drive transcription. Though some cytokines and growth factors activate multiple STAT proteins, some STATs are activated with considerable specificity [91]. STAT3 and src can interact independently leading to the phosphorylation of STAT3 [92]. Accumulating evidences suggest that activated STAT3 takes part in tumour progression in various cancers like breast, head and
neck, prostate and leukemia [93, 94]. STAT3 signalling has been well established in head and neck cancer [95-97]. STAT5, comprising 2 highly homologous proteins, STAT5a and STAT5b, is activated late in pregnancy by prolactin to promote terminal differentiation and milk production. Abnormal STAT5 activity is found in breast cancers as well as in head and neck cancer and in well-differentiated tumours [91, 98, 99]. STAT proteins control cellular processes, such as growth, apoptosis, transformation, inflammation, proliferation, and differentiation, metastasis, and angiogenesis [91].

**AP-1**

Agents activating NF-κB also activates the early response transcription factor, activator protein-1 (AP-1). AP-1 family are made up of homo/heterodimers of Jun or heterodimers of Jun-Fos proteins. Jun protein family include c-Jun, Jun B and Jun D. Fos family comprises of c-Fos, Fos B, Fra-1 and Fra-2. These proteins have basic region leucine zipper (bZIP) domains in their structure. c-Jun combines with c-Fos to form AP-1. AP-1 binds with specific gene promoters and mediates early gene expression thereby taking part in transcriptional regulation processes [100, 101]. AP-1 controls cellular differentiation, apoptosis and promote cell proliferation by activating cyclin D1. AP-1 activation is implicated in chemical carcinogenesis. AP-1 has a key part in tumour initiation, progression and metastasis [102]. Overexpression of c-Jun in breast cancer results in increased aggressiveness. This is indicated by augmented cellular motility, synthesis of ECM degrading enzyme and chemoinvasion.

**Cyclins**

The eventual result of all growth-promoting stimuli is the entry of quiescent cells into the cell cycle. The process of cell division involves series of synchronized events that makes up "cell division cycle". The mammalian cell cycle is divided into a series of sequential phases - G1, S, G2, and M. This sequential phase transitions occur in response to growth factor or mitogenic stimulation (Fig. 7). The DNA synthetic (S phase) and mitotic (M phase) phases are preceded by gap phases (G1, G2). Cell cycle progression is tightly regulated in normal cells. One set of molecules sense growth-promoting conditions and signal second set of molecules which control cell division. In addition, cells have signalling pathway that can sense unfavourable conditions for proliferation and block cell
division [103-106]. Mutations or amplification of molecules in these signalling pathways result in formation of abnormally increased proliferative cells, evidenced as cancer [107, 108].

Cell cycle progression is controlled by cyclin family and their catalytic partners, cyclin-dependent kinases (CDK). The G1-S and G2-M transitions are the main cell cycle check points in mammalian cells. The G1-S phase progression is regulated by cyclin D and E family. Cyclin D, consists of 3 subtypes, D1, D2 and D3, which interact with CDK4/6 and CDK2, respectively [108]. Overexpressed cyclin D1 is reported in breast, oesophagus, lung, liver, head and neck, colon and prostate [109-114]. The cyclin D1 gene is amplified in 20-50% of SCC, and its protein is overexpressed in 80% of SCCs [113]. Cyclin D1 expression is regulated by NF-κB and p53 [115]. Cyclin B1 with CDK1 aids G2-M transition.

**Fig. 7. Cell cycle Checkpoint [116]**

Overexpressed oncogenes that have been implicated in HNSCC & Breast cancer are

a) HNSCC: *EGFR*, members of the *ras* and *myc* gene family, *int-2*, *hst-1*, *cyclin D1* and *Bcl-2*.

b) Breast cancer: *PI3K*, *Cyclin D1*, *Bcl-2* etc.
2. Insensitivity to anti-growth signals

Antigrowth signals rendered by tumour suppressor genes and their products apply brakes to cell proliferation by 2 complementary mechanisms: they stimulate proliferating cells to enter the G0 (quiescence) phase; alternatively they induce cells to enter a post mitotic, differentiated pool and lose replicative potential. It is now clear that at molecular level they exert their effects on G1-S checkpoint of the cell cycle. Disruption or loss of function of tumour suppressor gene renders cells uncooperative to growth inhibition and mimics the growth-promoting effects of oncogenes leading to unregulated cell growth. Some of the important tumour suppressor genes which are altered in cancer cells are pRb, p53, TGF-β, β-catenin.

β-catenin

β-catenin is a key factor in WNT signalling pathway. WNT is a soluble factor that can induce cellular proliferation. It does so by binding to its receptor and transmitting signals that prevent the degradation of β-catenin, allowing it to translocate to the nucleus. β-catenin acts as a transcriptional activator in conjunction with another molecule, called Tcf [117]. APC (Adenomatous polyposis coli) a cytoplasmic protein regulate the intracellular levels of β-catenin. In quiescent cells which are unexposed to WNT, cytoplasmic β-catenin is degraded by a destruction complex. APC forms an integral part in this destruction complex. With loss of APC (in malignant cells), β-catenin degradation is prevented, and the WNT signalling response is continually activated. This leads to transcription of growth-promoting genes, such as cyclin D1 and myc [118].

p53: choice of response: DNA repair or cell death

p53, a well-conserved phosphoprotein, is one of the best known tumour suppressors. It has a main function in cancer pathogenesis [119]. It mediates G1 arrest, DNA damage repair and induction of apoptosis [120]. p53 which is recognized as the "guardian of genome", and regarded as "a cellular gatekeeper for growth and division" is vital to control cell growth and apoptosis [121, 122]. Normal p53 in unstressed cells has a short half-life. This short half-life is due to the association with MDM2, which marks p53 for destruction. However under stressed conditions due to DNA assault by hypoxia or radiation, p53 is modified post-transcriptionally and is released from MDM2. During the
process of being unshackled from MDM2, p53 is activated as a transcription factor. p53 elicits transcription of genes involved in cell cycle arrest and apoptosis. p53 mediates cell cycle arrest as a primitive response to DNA damage. It causes growth arrest before either DNA replication in the G₁ phase or before mitosis in the G₂ phase. Such a pause in cell cycle gives the cells "breathing time" to repair damaged DNA [108]. Hence, p53 is considered as an important regulator of DNA repair that ensures genomic integrity. If the damaged DNA is repaired successfully, p53 upregulates transcription of MDM2, which then downregulates p53, and the cell cycle block is relieved. If during the pause the damaged DNA is not repaired successfully, p53 induces cell death by triggering apoptosis. It does so by inducing apoptosis inducing genes such as Bax [123]. The importance of p53 in carcinogenesis is attested by the fact that more than 70% of human cancers have a defect in this gene and the remaining have defects in genes up-stream or down-stream of p53. Homozygous loss of the p53 gene is found in every type of cancer, including carcinomas of the lung, colon, and breast, the three leading causes of cancer deaths.

3. Evading apoptosis

Apoptosis is a highly regulated, evolutionarily conserved pathway of cell death. Apoptosis or programmed cell death is involved in maintenance of tissue homeostasis by providing a controlled cell deletion to balanced cell proliferation [124,125]. Resistance to apoptosis is considered as a hallmark of most types of cancer. The morphological changes occurring in apoptotic cells include: membrane blebbing, shrinking of cytoplasm, aggregation of chromatin at the nuclear membrane and condensation of nucleus, fragmentation of cell into smaller bodies and formation of membrane bound vesicles-apoptotic bodies (Fig. 8).
At the molecular level, apoptosis typically occurs in a succession of key steps, including induction, activation, and execution. Apoptosis is carried out by cysteinyI aspartate-specific proteases called caspases. They disassemble the cell in systematic manner by cleaving many cellular substrates. Caspases are “engine of the apoptotic pathway”. Caspases-8, 9 and 10 are the initiator caspases. They activate the downstream effector caspases -3, 6 and 7. The initiator caspases are activated classically by two different pathways involving different caspases as follows (Fig. 9): The death receptor (extrinsic) pathway and the mitochondria (intrinsic) mediated pathway.
Fig. 9. Molecular mechanism in apoptosis [126]

The death receptor-mediated pathway

The extrinsic pathway is initiated by cell surface death receptors that transmit apoptotic signals, when activated by ligands like TNF-α and FasL. Once these receptors are activated, they oligomerize and form scaffolding complexes that classically recruit caspase-8. This activated initiator caspase then cleaves the effector caspases, particularly caspase-3, which culminates in apoptosis. Stimulation of apoptosis through this pathway is rapid [127].
Mitochondria-mediated pathway

Mitochondria serve as death signal integrators in apoptosis [128-130]. Cellular stress activates Bcl-2 family members which play key roles in initiating mitochondrial dysfunction [131]. This family includes pro-apoptotic factors (Bax, Bid, Bak, or Bad) that promote apoptosis and anti-apoptotic factors (Bcl-Xl and Bcl-2) that prevent apoptosis. Mitochondrial membrane disruption culminates in cytochrome c leakage. This appears to be a crucial event. Bcl-2 is associated with the outer mitochondrial membrane. It regulates cell survival by inhibiting mitochondrial permeability transition (MPT) [132]. Bcl-2 dimerize with Bax and neutralises the proapoptotic effect of Bax. When Bax is released from this heterodimer complex it acts as a dominant- negative inhibitor of Bcl-2, promoting MPT pore (MPTP) formation [133, 134]. Opening of MPTPs releases cytochrome c which is localized in the cristae of inner mitochondrial membrane. Cytochrome c plays a pivotal role in electron-transport system. Cytosolic procaspase-9, oligomerized Apoptotic protease activating factor-1 (Apaf-1), dATP and cytochrome c form a massive supramolecular apoptososme complex. The N-terminal of Apaf-1 and the prodomain of procaspase-9 interact in 1:1 stoichiometric ratio to form a complex [135-137] leading to activation of caspase-9.

The mitochondrial-activated caspase-9 cleaves and activates caspase-3, the “central executioner of apoptosis”. Activated caspase-3 induces morphological and biochemical changes of apoptosis (132, 138-140). One of the important substrate of activated caspase-3 is Poly (ADP-ribose) polymerase (PARP). PARP cleavage occurs early in the apoptotic response as a result of caspase-3 activity [141]. PARP is a highly conserved nuclear enzyme present in higher eukaryotes. It binds with DNA and recognizes DNA strand breaks. It is implicated in DNA repair. PARP is also involved in cellular apoptotic response [142]. The enzyme adds poly ADP-ribose, in an ATP and NADP dependent manner at single strand DNA break sites and signals various proteins in response to DNA damage. It directly interacts with DNA polymerase. Cleavage of PARP correlates well with chromatin condensation and precedes DNA fragmentation.
In cancer cells, apoptosis is disturbed at many spots. Tumour cells evade apoptosis by 2 ways:

- Upregulation of inhibitors of apoptosis.
- Blocking mitochondrial apoptotic pathway, loss of Apaf-1, thereby developing resistance to p53-induced apoptosis.

### 4. Limitless replicative potential

Normal cells have limited replicative potential and after a certain number of cell divisions they stop dividing and enter into senescence [143]. However, disabling pRb or p53 tumour suppressor genes have been shown to enable cells to continue multiplying for additional generations until they enter a second phase called crisis. At this stage cells undergo massive cell death. Tumour cells avoid this cellular senescence by activation of the enzyme telomerase, which can maintain normal telomere length. Telomeres are repeat sequences at the end of chromosomes that protect the genetic stability during DNA replication. Telomeres are lost during each cell division, and this increases chromosomal instability and cellular senescence. Telomerase is absent in somatic cells. In contrast, telomere preservation is observed in all cancers. This is because of upregulation of telomerase in 85-95% of cancers. Only few cancers utilize other mechanisms.

### 5. Tissue invasion and metastasis.

Metastasis is responsible for 90% of cancer mortality [144]. In this process, cancer cells migrate from the tissue of origin to other distant sites through blood flow and form new malignant lesions in other organs [145]. In cancer, invasion-metastasis cascade is an intricate, multistep process. It involves leakage of neoplastic cells from a primary tumour (local invasion), intravasation into the systemic circulation, survival during transfer through the vasculature, extravasation into the parenchyma of distant tissues, the formation of micrometastases and finally the outgrowth of macroscopic secondary tumours [146] (Fig. 10a, 10b).
Fig. 10a. Neoplastic invasive growth [147]

Epithelial cells → Proliferation → Migration, invasion → Metastasis

Blood vessel
Survival

Fig. 10b. Principal steps in metastasis [148]
Formation of metastatic foci involves proteolytic degradation of ECM, modifications in cell to cell and cell to ECM interactions and migration of the cancer cell through the basement membrane [145, 149]. ECM degradation is one of the hallmarks of tumour invasion and migration. During cancer progression, the balance between the ECM degrading enzymes and their inhibitors is disturbed due to the overexpression of proteases such as matrix metalloproteinases (MMPs) [150]. MMPs degrade ECM and basement membrane and help malignant cells to invade tissues.

**MMPs**

The MMPs, also called matrixins are \( \text{Zn}^{2+} \) dependent endopeptidases that hydrolyze components of the ECM. MMP group of proteins produced by carcinoma and stromal cells consist of 23 members. Among them, 17 are secreted as soluble enzymes and 6 are membrane bound enzymes [151]. These include collagenases (MMP-1, MMP-8, MMP-13 and MMP-18), gelatinases (MMP-2 and MMP-9), stromalysines (MMP-3, MMP-10 and MMP-11), matrilysines (MMP-7 and MMP-26), membrane bound MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25), and others [152] (Fig. 11, 12).

**Fig. 11. Various Functions of MMPs in Tumour Microenvironment [153]**
Fig. 12. Modulation of the Tumour Microenvironment by MMPs [153]

MMP expression is regulated by hormones, growth factors and cytokines [154]. MMPs, particularly MMP-2 and MMP-9 play a crucial role in the development of many cancers and are expressed in various malignant tumours [155]. MMP-2 degrades gelatins, collagen IV, V, VII, elastin and proteoglycan. MMP-9 participates in basement membrane remodeling and cell migration. The basement membrane components, collagen IV and laminin, are degraded by active MMP-9.

Expression of MMP-2 and MMP-9 is modulated at transcriptional and post-transcriptional levels [156,157] with MMP-2 being regulated at the post-transcriptional level via interaction with the tissue inhibitor of metalloproteinase-2 (TIMP-2) [158]. MMP-9, in contrast, is controlled at the transcriptional level by the transcription factors which interact with the MMP-9 promoter like Ets, AP-1, and NF-κB [156, 159, 160]. Inductions of mitogen-activated protein kinase (MAPK) signalling or PI3K signalling are also involved in the expression of MMP-9 [150, 161]. The enhanced expression of MMP-9 is often associated with human cancer invasion and metastasis [162, 163]. The MMPs also aid epithelial-to-mesenchymal transition (EMT) associated with malignant behaviour.
by cleaving the cell-adhesion molecule E-cadherin and liberating TGF-β. Both MMP-2 and MMP-9 are thus closely linked to the metastatic potential of tumours [157-161, 164]. They also share the common feature of forming complexes with tissue inhibitors of matrix metalloproteinases (TIMPs), to control their local activities.

**Endogenous MMP Inhibitors**

TIMPs are endogenous inhibitors of MMPs, which associate in 1:1 ratio. TIMPs expression is regulated during development and tissue remodelling. Overexpression of TIMPs decreases the metastatic ability of tumours and inhibits tumour growth, while depletion of TIMPs results in formation of more tumourigenic, invasive and metastatic cells [165]. MMP-TIMP combinations form pro-MMP-TIMP complexes (e.g. complexes of pro-MMP-9-TIMP-1 and pro-MMP-2-TIMP-2). MMP-9 is typically processed from 92 to 86, 82, 65, and 50 kDa by TIMP-1 with which it forms proenzyme complex (pro-MMP-9-TIMP-1). N-terminal domain of TIMP-1 binds to the active site cleft in MMP-9 and inhibits MMP-9. TIMP-2 (Fig. 13) has been found to block tumour cell invasion both in vitro and in vivo and may act as metastasis suppressor gene.

**Fig. 13. TIMP-2 mediated MMP inhibition [166]**
6. Sustained angiogenesis

Tumour angiogenesis is the formation of a network of blood vessels that infiltrate the cancerous growth, supplying the cancer cells nutrients and oxygen and removing waste products. For most solid tumours, angiogenesis is essential for tumour growth and metastasis [167]. Tumour angiogenesis begins when the cancerous tumour cells release molecules that send signals to surrounding normal host tissue. Some proangiogenic regulators are growth factors (VEGF, EGF, TGF-α, TNF-α, keratinoeyte growth factor, IGF-I, bFGF, PDGF), cytokines-interleukin (IL-1α, IL-6, IL-8) and smaller molecules (adenosine, prostaglandin E). Among these molecules, VEGF, bFGF and IL-8 are most important for sustaining tumour growth.

Vascular endothelial growth factor (VEGF)

VEGF is the predominant stimulator of angiogenesis [168, 169], where it mediates vasculogenesis, angiogenic remodeling, and angiogenic sprouting [170]. Normally tissue vasculature is mediated by VEGF expression [171]. VEGF expression is induced by tumour specific factors like oncogenes [e.g., ras, src, EGFR] and hypoxia (Fig. 14).

Oxygen tension is vital for angiogenesis in tumour growth [172]. VEGF mRNA expression is induced in low oxygen tension that takes place in poorly vascularized tumours [173]. Binding of VEGF to VEGF receptor results in receptor dimerization, ligand-dependent receptor tyrosine kinase phosphorylation and activation of intracellular signalling. This signalling pathway involves endothelial cell proliferation, migration, survival, sprouting, and tube formation, as well as upregulation of molecules involved in degradation of the ECM [169, 174] (Fig. 14). Endothelial cell activation results in MMP and urokinase plasminogen activator secretion [175]. Degradation of ECM allows proliferating cells to migrate towards the growth factor source. VEGF also mediates the effects of other angiogenic molecules and is therefore considered as crucial player in control of tumour angiogenesis.
7. Inflammatory microenvironment

Inflammation is a vital part of the tumour microenvironment. The tumour microenvironment comprises of immune, stromal, tumour and inflammatory cells. It synthesise growth factors, cytokines, and adhesion molecules. The blazing inflammation in this tumour microenvironment underwrites to proliferation and survival of malignant cells, angiogenesis, metastasis and subversion of adaptive immunity. 15% of cancers are because of inflammatory etiologies [177]. Normally physiologic inflammatory response arises when tissue is injured. Inflammatory cells secrete cytokines and chemokines and reparative inflammation occurs. However, chronic pathological inflammation results when there is continuing occurrence of stimulus, like tumour cells. The resulting extended inflammatory cytokine exposure promotes tumour growth through the induction of angiogenesis and other events favourable to tumour invasion and metastases [178]. Key instigators of the inflammation-mediated tumour progression are transcription factors, cytokines, chemokines such as NF-κB, STAT3, COX-2, TNF-α, IL-1β, IL-4, IL-6 and IL-8, [179-182].

Cytokines control growth of stromal and tumour cells. Cytokines secreted by cancer cells create optimal growth conditions in the tumour microenvironment, while
those produced by stromal cells control malignant cell behaviour [179]. Cytokines induced by hypoxia in progressive cancers, include VEGF, TNF, IL-1, and IL-6 [178].

NF-κB and STAT3 converge in numerous oncogenic signalling pathways (184). Lee et al [183] showed that the maintenance of NF-κB activation in tumours requires STAT3. STAT3 regulates the ‘danger signal’ for increasing antitumour immunity [184] and is involved in carcinogenesis. IL-6 is associated with activation of NF-κB and STAT3. It has growth-promoting and antiapoptotic activity [185,186]. The NF-κB –IL-6–STAT3 cascade plays a major role in metastasis.

Cyclooxygenase-2 (COX-2), the rate-limiting enzyme in prostaglandin synthesis, is induced in many cells by inflammatory mediators [187]. Cyclooxygenases convert arachidonic acid to prostaglandins. COX-2 is regulated by mitogens, tumour promoters, cytokines and growth factors. It is overexpressed in malignant conditions of colon, liver, pancreas, breast, lung, bladder, skin, stomach, head and neck, and oesophagus [188]. COX-2 expression in animal cancer models is associated with control of tumour cell growth, inhibiting apoptosis, cell migration, invasion, metastasis and suppression of immune response (187). COX-2 expression is higher in metastatic tumours [189] and is associated with lymph node and distant metastasis [190-192]. High COX-2 levels correlate with increased expression of VEGF [193], IL-11 [194], and IL-8; [195] in breast cancer cells. COX-2 overexpression increases the motility and invasive properties of breast cancer cells [196].

Interleukin-1 (IL-1), a pleiotropic cytokine, has many roles in physiological and pathological states. It is upregulated in many tumour types and is implicated in tumour progression via the expression of metastatic and angiogenic genes and growth factors. IL-1 includes a family of closely related 2 major agonistic proteins, IL-1α and IL-1β. They differ in their compartmentalization. IL-1β is active in secreted form. IL-1α is active in cell-associated form. IL-1β, secreted by malignant cells, induces inflammation and endorses invasiveness [197]

Solid tumours in which IL-1 is upregulated include breast, colon, lung, head and neck cancers, and melanomas. IL-1 promotes tumour growth by induction of pro-
metastatic genes, angiogenic proteins and growth factors such as like MMPs, VEGF, IL-8, IL-6, TNF-α, and TGF-β [197, 198-200].

Tumour necrosis factor (TNF) mediates acute and chronic inflammation. Aberrant TNF expression is detected in breast, prostate and ovarian carcinomas [201]. TNF, IL-6, and IL-8 promote tumour growth by inducing VEGF expression [202, 203].

Interleukin-6 (IL-6) regulates immune and inflammatory responses. Elevated expression of IL-6 in multiple epithelial tumours is an important prognostic factor for breast cancer progression. IL-6 binds to a heterodimeric receptor. This leads to activation of the Janus kinase and stimulation of multiple pathways involving MAPKs, PI3Ks, STATs, and other signalling proteins. IL-6 induces intracellular signalling through STAT3 [204].

Interleukin-8 (IL-8), a chemotactic factor for leukocytes, contributes to cancer progression as it acts as mitogenic, angiogenic, and motogenic factor. IL-8 expression is controlled by several tumour microenvironment factors, such as hypoxia, nitric oxide and cell density. High levels of IL-8 and its receptors are found in malignant breast tumours than in benign breast tissue [205]. It is associated with promoting invasion and metastasis of breast cancer cells [206] where it acts along with MMP-9. IL-8 gene transcription is induced by NF-κB [207, 208]. IL-8 also mediates and maintains epithelial mesenchymal transition (EMT) along with TGF-β [209].

Tumour growth factor-β (TGF-β) is a secreted protein that exists in 3 isoforms: TGF-β1, TGF-β2 and TGF-β3. TGF-β stimulates mesenchymal cell growth. It induces EMT of mammary epithelial cells. EMT controls tumour progression [210-212]. In many final cancer stages, TGF-β stimulates invasive behaviour of cancer cells, promotes neo-angiogenesis and aids cancer cells to escape immune surveillance.

Interleukin-4 (IL-4) is involved in several human diseases including autoimmunity, allergies, and cancer. IL-4 receptor α (IL-4Rα) is found to be expressed on solid human tumours including malignant melanoma, breast carcinoma, ovarian carcinoma, mesothelioma, glioblastoma, renal cell carcinoma, head and neck carcinoma, and AIDS-associated Kaposi’s sarcoma [213].
The overall scenario of integrated signalling circuit in cancer cell is depicted in Fig.15.

Fig. 15. Integrated Signalling Circuit of cancer cell [36]

Many of the above said signalling molecules are involved in the development and pathology of HNSCC and breast cancers and are therefore focused in the present research work.
The cancer therapy

Despite great advances in understanding cancer biology, oncologists have made very petite progress in cancer prevention and treatment. In another side, WHO projects a doubling of cancer incidence in the next 21 years.

There are more than 100 distinct types of cancer. Currently, advances in biomedical sciences and technology have enabled the contemporary medicine to provide sophisticated cancer therapy varying from surgery to molecular targeted therapy. However, cancer is still among the major culprits of health in humans throughout the world.

The cancer therapies available are:

- Local therapy: Surgery, Radiation therapy
- Systemic treatment: Chemotherapy, Hormonal therapy, Immunotherapy, Targeted therapies, Transplantation
- Non-conventional therapy
- Other treatment methods: Angiogenesis inhibitors, Cancer vaccines, Cryosurgery, Hyperthermia, Laser treatment, Photodynamic therapy for cancer.

General types of treatment for breast cancer:

Local therapy: Surgery and radiation therapy are examples of local therapies.

Systemic therapy: Chemotherapy, hormone therapy, and targeted therapy are systemic therapies. Drugs given to treat breast cancer include anthracyclines and taxanes in combination with fluorouracil (5-FU), cyclophosphamide or carboplatin. Hormone therapy utilises drugs like Tamoxifen, Toremifene, Fulvestrant and Aromatase inhibitors.

Adjuvant and neoadjuvant therapy: Adjuvant therapy prevents the relapse of cancer after surgery. Systemic therapy and radiation are used in adjuvant therapy. Targeted therapy uses drugs that specifically target signalling molecules involved in breast carcinogenesis. Examples for targeted therapy drugs include Everolimus, Lapatinib, Trastuzumab, pertuzumab.
General types of treatment for Head and Neck Cancer

Treatment for laryngeal cancer includes Surgery, Radiation therapy, Chemotherapy, Photodynamic therapy, Targeted therapy. Advanced laryngeal cancers are treated with concurrent chemotherapy. Chemotherapeutic agents for treating HNC include combination of paclitaxel and carboplatin. Some targeted therapies used in HNSCC include cetuximab, bevacizumab and erlotinib.

The chemotherapeutic window

Cancer chemotherapy had its origins one century ago, with the establishment of Paul Ehrlich's side-chain/receptor theory, where he first proposed the concept of targeted therapy against human diseases [214]. Cancer chemotherapy became practical in the early 1940s due to the breakthrough research of Louis Goodman and Alfred Gilman. They used nitrogen mustard for cancer treatment [215]. Nonetheless, despite decades of research, we have made little progress in changing cancer mortality statistics, with cancer still accounting for about 25% of all deaths [216, 217].

Cancer drug discovery challenges

Identifying novel, potent therapies for cancer is hindered by numerous factors inherent to cancer drug research, such as chemoresistance and tumour heterogeneity. Tumour heterogeneity poses a hurdle to drug discovery as well, as it is difficult to target specific upregulated or altered proteins in the face of wide molecular variability among tumours and the diversity of the cells within the tumour [218]. A major difficulty in treating cancer is dose-limiting and poor quality-of-life side effects, which limits the amount of chemotherapy that can be administered. These factors greatly complicate target based drug discovery.
Significance of multitargeting over monotargeting

The underlying molecular bases for cancer are more complex and frequently involve many targets [219]. A specific mono-target-based drug, once called smart drugs and magic bullets are inadequate to normalize the diverse molecular targets. Moreover mono-targeting drugs have multiple side effects [220]. Hence using multi-target drugs in cancer treatment has become essential in therapeutic regimens. Many natural products utilised in cancer treatment exhibit multiple targeting ability. Hence they are preferred over monotargeted drugs [221].

The concept of chemoprevention

Cancer chemoprevention utilises natural/synthetic chemicals to suppress, retard or inverse carcinogenesis [222]. The word ‘cancer chemoprevention’ was 1st introduced by Dr. Michael Sporn [223]. The chemopreventive agents are grouped as blocking and suppressing agents. Blocking agents inhibit cancer occurrence either by blocking the interaction between carcinogen and intracellular macromolecules or by deactivating and clearing the carcinogen. Suppressing agents control or reverse the damage caused by a carcinogen. Previous studies hypothesize that chemoprevention reduce cancer and death incidence [37]. Thus chemoprevention is a promising anticancer approach. They exhibit less side effects and toxicity and are easily available.

Dietary agents as chemopreventive agents

One of the candidate approaches to discover new drugs with multitarget ability is to use natural compounds (or “nutraceuticals”) that are traditionally used for centuries in various parts of the world to treat different ailments (Fig. 16).
Almost 74% of the drugs approved between 1981 and 2002 for cancer were either natural products or analogs or mimics of natural products [224]. In addition to their multitargeting ability, components of dietary or medicinal plants possess good chemopreventive properties. It is estimated that modifying diet can result in prevention of more than 2/3rd of cancers [225]. Hence chemoprevention by edible phytochemicals is now considered to be an inexpensive, readily applicable, acceptable, and accessible approach to cancer control and management [226]. Many reports have indicated that dietary constituents, principally phytochemicals, can prevent carcinogenesis at multiple steps [220]. Some of the active dietary phytochemicals that show anticancer activities include curcumin, diallyl sulfide, isothiocyanates, S-allyl cysteine, allicin, lycopene, capsaicin, diosgenin, 6-gingerol, resveratrol, ellagic acid, ursolic acid, betulinic acid, beta carotene, silymarin, anethol, catechins, eugenol, flavonoids, isoeugenol, dithiolthionesindole-3-carbinol, isoflavones, protease inhibitors, saponins, phytosterols, inositol hexaphosphate, Vitamin C, Vitamin E, D, limonene, lutein, folic acid, selenium,
and dietary fibers [26]. One of these promising dietary chemopreventive compounds with established effects in many tumour models is curcumin.

**Curcumin**

Turmeric (*Curcuma longa*) is a plant indigenous to Southeast Asia and is widely used in Indian subcontinent as spice. It is used as a traditional remedy in "Ayurvedic medicine" that dates back over 5,000 years [227]. It is used to treat various ailments such as inflammatory, neoplastic and other conditions for many centuries. It contains a class of compounds known as the curcuminoids, comprised of curcumin, demethoxycurcumin and bisdemethoxycurcumin [37]. Curcumin [diferuloyl methane; 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] an yellow pigment, is a naturally occurring phenolic diarylheptanoid isolated from the dry rhizome of turmeric [228] (Fig. 17).

**Fig. 17. Three main classes of curcuminoids in turmeric [37]**

![Curcumin structures](image)

Biological activities of turmeric are mostly due to its major bioactive constituent, curcumin. Curcumin and turmeric products are certified as safe by FDA in USA, FAO, WHO [109].

This compound has attracted considerable attention as it exhibited a wide spectrum of pharmacological properties [229], like antioxidant [230-232], anticancer [227, 233-236], anti-inflammatory [88, 224], antimutagenic activities and inhibited tumourigenesis
in various tissues in animal models [237, 238]. It is also tested to treat various diseases such as diabetes, allergies, asthma, arthritis, atherosclerosis, neurodegenerative diseases, and other chronic illnesses like cancers on account of its manifold clinical applications [30].

Curcumin, a pleiotropic molecule, has multitargeting ability (Fig. 18). These include transcription factors (e.g., NF-κB, STAT3, AP-1, Nrf-2, PPAR-γ, and HIF-1), receptors (e.g., HER-2, IL-8, and CXCR-4), kinases (e.g., EGFR, ERK, JAK, and AAPK), cytokines (e.g., TNF, IL, MIP, and MCP), enzymes (e.g., MMP, iNOS, GST and ATPase), and growth factors (e.g., EGF, NGF, HGF, and PDGF) [239] (Fig. 19).

Fig. 18. Curcumin representation of multi and monotargets [241]
Numerous studies have demonstrated that curcumin targets several steps in carcinogenesis pathway (Fig. 20) [108]. Curcumin is in phase I, II clinical trials for cancer chemoprevention and chemotherapy. Curcumin exerts its remarkable cytotoxic activity and apoptosis inducing ability upon a variety of cancer cell lines by modulating the activities of numerous transcription factors, growth regulators, adhesion molecules, apoptotic genes and cellular signalling molecules [240]. It is studied for its chemopreventive property in colon, breast, prostate, oesophagus, lung and oral cancers [241]. It mediates anti-inflammatory effects through the suppression of NF-κB activation, antiproliferative effects by suppressing cyclin D1 and anti-apoptotic gene products [242,
inducing cytochrome c release, activating caspases [244, 245] and p53 [246] and anti-angiogenic effects through the down-regulation of VEGF [224, 247].

Thus curcumin has multifaceted functions in influencing the expression of proteins that are involved in cellular proliferation, inflammation, adhesion, malignant transformation, metastasis, etc. Pro-apoptotic effects of curcumin are central to its chemopreventive efficacy.

**Fig. 20. Key oncogenic cell signalling targets by curcumin [108]**

Blunt–head lines indicate downregulated molecules, arrow-head lines indicate upregulated molecules by curcumin.
Despite promising findings, the *in vivo* application of curcumin is limited because of its low pharmacokinetics. This necessitates search for new curcumin analogues having improved pharmacological properties.

Previous research confirms the versatility and flexibility of curcumin for structural modifications. Based on a simple pharmacophore model, using standard drug design concepts, several analogs of curcumin have been prepared and screened for their therapeutic potential [249].

Reports have shown that many curcumin analogs are well known for their anti-tumour and antiproliferative activities [250] and thereby induce apoptosis in various cancers [251], yet mechanisms underlying these effects are not well understood.

The general structure of curcumin i.e., the conjugated chain with an adjacent phenolic group has the general property to act as a potential antioxidant and anticarcinogenic compound. With the basic structure of curcumin, a new curcuminoid was synthesized with similar structure but different substitute. This curcumin analog (BDMC-A) is an ortho isomer of bisdemethoxy curcumin (BDMC). BDMC is the most active curcuminoid isolated from *Curcuma longa* [252]. Our compound, BDMC-A, has antitumour, antimutagenic [253] and antioxidant [249] properties. The protective effects of BDMC-A in alcohol and ΔPUFA mediated oxidative stress [254], circulatory lipid profiles [255], hyperlipidemia [256], MMPs [257] in experimental rats have already been reported from our lab. BDMC-A has also been analysed for the anticarcinogenic effect in DMH-induced colon cancer [258]. Our present study is aimed to delineate the molecular mechanism underlying anti-carcinogenic inducing ability of BDMC-A in breast and laryngeal cancer cell lines and to compare its multitargeting efficacy with that of curcumin.