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MOLECULAR TARGETS FOR CANCER PREVENTION AND THERAPY

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Summary

Cancer is caused by accumulation of genetic damage in a susceptible cell. Majority of cancer causing mutations affect proteins that regulate essential cellular functions such as cell proliferation, cell cycle, differentiation, angiogenesis and apoptosis. Dominant mutations convert proto-oncogenes to oncogenes, and recessive mutations inactivate tumor suppressor genes. Many of the genes that are altered in human cancer define molecular pathways that are central to the control of the above processes. These pathways are not strictly linear but, rather, constitute molecular networks. The pathway of growth factors and cell cycle include Tyrosine Kinase receptors and downstream signaling via phospholipase C, Ras, PI-3 kinase, cdks. The dysregulation of these signals in tumor cell leads to multiple cellular changes. Therapeutic approaches include monoclonal antibodies against growth factors receptors, antisense oligos against key target proteins, enzyme inhibitors, antiangiogenic therapy, apoptotic triggers/ activating death signaling pathways. Nutrients such as Vitamin D, Selenium, folate, curcumin, flavonoids, etc. also influence the above molecular targets and can help in cancer prevention.

Keywords: Cancer prevention, cancer therapy, molecular targets, signaling

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Introduction

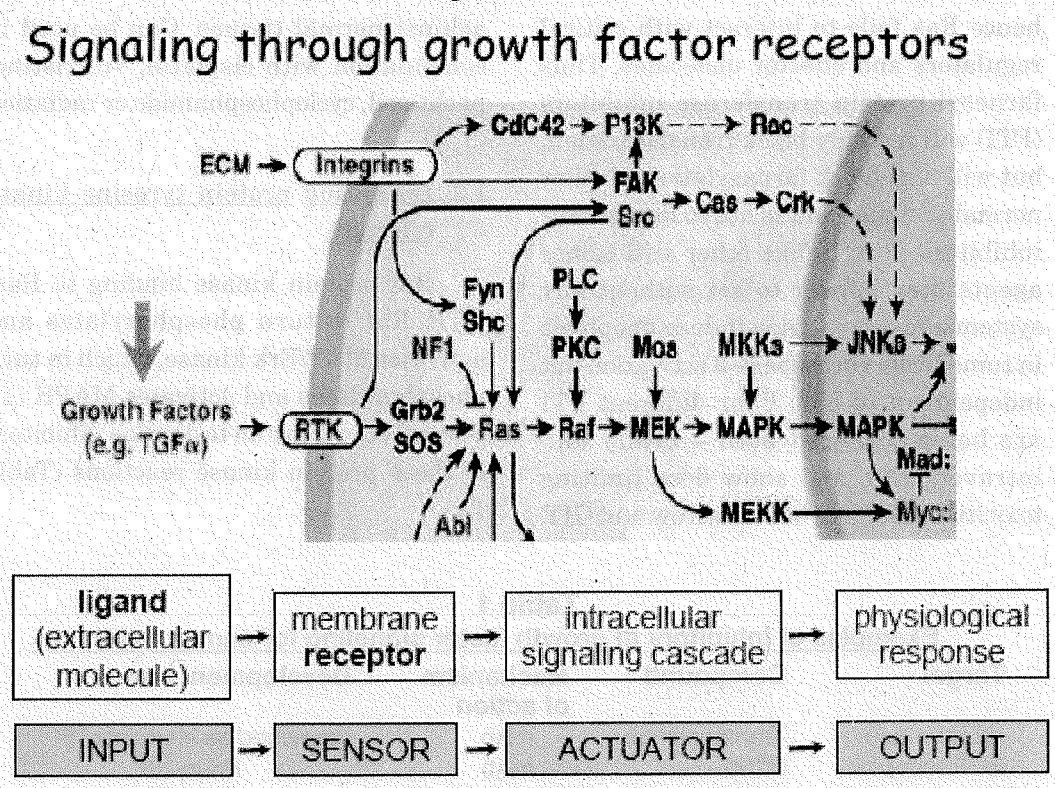
Cancer is a disease of multiple and changing genetic alterations that must be attacked with therapies having different mechanisms of action. Rational molecular approaches for anticancer therapies must be developed to control cell proliferation, cell cycle, angiogenesis, apoptosis, but these therapies/agents should have lethality to tumors without overt systemic toxicity. Signaling pathways that drive cell proliferations are closely related with tumor malignancy. Components of these pathways are encoded by oncogenes such as PDGF-like ligand Sis, Tyr kinase Src and HER-2/C-Neu and GTP-binding switch Ras. Mutations in key components lead to constitutive activation of these pathways and to proliferation.

I. Growth factor signaling

Signaling pathways are initiated with the binding of a ligand such as PDGF, EGF, EGF-like ligand (TGF-α) or IGF to its transmembrane receptor. Ligand binding induces the dimerization of receptor subunits promoting autophosphorylation of the receptor and recruiting a variety of intracellular docking proteins such as Grb2, Shc to the plasma membrane. From these docking proteins subsequent signals emanate. Ras serves as a molecular switch in the plasma membrane that alternates between the inactive GDP-bound state and an active GTP-bound state. Normally Ras is bound to GDP, but on recruitment of Sos to the plasma membrane, Sos binds Ras-GDP and facilitates release of GDP. GTP which is highly abundant in cells and binds to Ras. Ras-GTP adopts a conformation that permits interaction with downstream effector molecules such as Raf, which activates MAP kinase cascade (1). The dysregulation of signals in tumor cells leads to multiple cellular changes including alterations in DNA synthesis, lipid metabolism, cellular morphology, cell adhesion properties and gene expression (Figure 1).

The signaling mechanism has given therapeutic agents in the treatment of cancer which include anti-estrogens, anti-androgens, agonists of gonadotropin releasing hormones and stem cell growth factors. Therapeutic approaches include molecular antibodies (mAbs) against extracellular domain of receptors, antisense oligonucelotides to key target proteins and enzyme inhibitors (Table 1). Inhibition of HER-2 yielded the first cancer therapeutic agent based on growth factor signaling. Unlike other members of EGF – receptor family, HER-2 has no known ligand, its expression is upregulated in 25-30% of human breast cancer. This upregulation promotes HER-2 heterodimerization with other members of EGF receptor family, as well as HER-2 homodimerization, resulting in constitutionally active tyrosine kinase. The mAb against HER-2, Transtuzumab is directed against extracellular domain of HER-2. It inhibits proliferation by several mechanisms. The mAb upregulates P27 Kip inhibitor of some

Figure 1



cyclin-dependent kinases, it also accelerates the internalization and degradation of HER-2, reducing cellular level of tyrosine protein kinase. It includes immune mediated effects such as cell mediated cytotoxicity and complement fixation. Along with cisplatin, doxorubicin, paclitaxel it shows enhanced anti tumor activity but with doxorubicin, may have higher cardiotoxicity. Therapeutic antibodies have also been developed against EGF receptor for eg. C-225 and E7.6.3, which are being clinically evaluated. Inhibitors of the intracellular tyrosine kinase for eg. ZD-1839, a competitive inhibitor of ATP are in clinical trials (2).

Drug Targets include the following

I.1. GTPase switch

Mutated forms of Ras have been found in many solid tumors as well as leukemia. The agents in clinical trials are based on regulating Ras gene expression or by inhibiting protein farnesylation. Ras proteins carry an essential lipid moiety – a farnesyl group at carboxy termini. Inhibitor of Ras farnesylation blocks Ras

localization to the plasma membrane and hence Ras fails to interact with critical regulators and effector molecules. Thus farnesyl protein transferase inhibitors (FTI) will not only block transformation, but will also block farnesylation of other normal proteins, and so not so Ras specific inhibitors. But, unlike other anti tumor agents they appear to act without any systemic toxicity – they induce apoptosis in tumor cells via caspase-3 activation but independent of p53. Four different FTI are being explored both – orally and intravenously, and show dose limiting toxicities involving bone marrow and GIT. FTIs can have antiproliferative effect against normal tissues. Can be used in combination with cisplatin, vincristine, paclitaxel, cyclophosphamide or radiation (3, 4).

I.2. Inhibiting protein tyrosine kinase effectors

Raf protein kinase binding to Ras-GTP. Raf in turn phosphorylates and activates MAP/Erk kinase, which in turn phosphorylates and activates MAPK (1). Several efforts are on to develop inhibitors of these protein kinase reactions (Table 1)

Table 1
Examples of inhibitors of growth factor signaling for cancer treatment

Target	Compound	Mechanism of action	Development status
HER2/c-neu	Transtuzumab	mAb	Launched as Herceptin™
EGF receptor	C225	mAb	Phase III
	E7.6.3	mAb	Preclinical
	ZD-1839	Kinase inhibitor	Phase II
	CP-358,774	Kinase inhibitor	Phase II
	PD-168,393	Kinase inhibitor	Preclinical
PDGF receptor	SU-101	Kinase inhibitor	Phase III
IGFR	AS ODN	Antisense	Preclinical
Ras	ISIS-2503	Antisense	Phase II
	R115777	FTI	Phase II
	SCH66336	FTI	Phase II
	L-778,123	FTI	Phase I
	BMS-214662	FTI	Phase I
Raf	ISIS-5132	Antisense	Phase II
	ZM336372	Kinase inhibitor	Preclinical
	L-779,450	Kinase inhibitor	Preclinical
MEK	PD-184352	Kinase inhibitor	Preclinical
	U0126	Kinase inhibitor	Preclinical
PKC	ISIS-3521	Antisense	Phase II
	CGP41251	Kinase inhibitor	Phase II
	UCN-01	Kinase inhibitor	Phase I
PI3'-Kinase	LY294002	Kinase inhibitor	Preclinical

I.3. Blocking lipid mediated signaling

Activation of GF receptor is also associated with changes in phospholipid metabolism. The phosphorylated residues on the intracellular domain of these receptors bind phospholipase C which then cleaves membrane phospholipids. One of these breakdown products, diacylglycerol activates PKC-α, which has been implicated in cell proliferation and tumorigenesis (5). PKC-α has been found to be increased in breast tumor. Both antisense inhibitors of PKC- α and inhibitors of PKC kinase activity are in clinical trials. The kinase inhibitors are derivatives of staurosporine. Therapies developed against growth factor regulated proliferation pathways are reaching the clinic to be tested. Most of these compounds do not act solely on tumor tissue, and hence their toxicities must be managed.

II. Cell cycle and checkpoint controls

Components of cell cycle machinery are frequently altered in human cancer. Central players are cell cycle dependent kinases (cdks) which help in cell cycle phases. Cdks are controlled by their association with cyclins and cdk inhibitors, by their state of phosphorylation and by ubiquitin mediated proteolysis. As malignant cells evolve both genetic and epigenetic mechanisms affect the expression of cell cycle regulator proteins causing overexpression of cyclins and loss of expression of cdk inhibitors – major consequence is deregulation of cdk activity providing cells with a selective growth advantage. Specific kinase inhibitors can block cell cycle progression and induce growth arrest (6). Failure of cell cycle arrest or apoptotic responses in malignant cells in response to cellular damage and ensuing instability may lead to emergence of malignant clone. Many anticancer agents act at multiple steps in the cell cycle and their effects may be cytostatic or cytotoxic depending on status of cell cyle of the target cell.

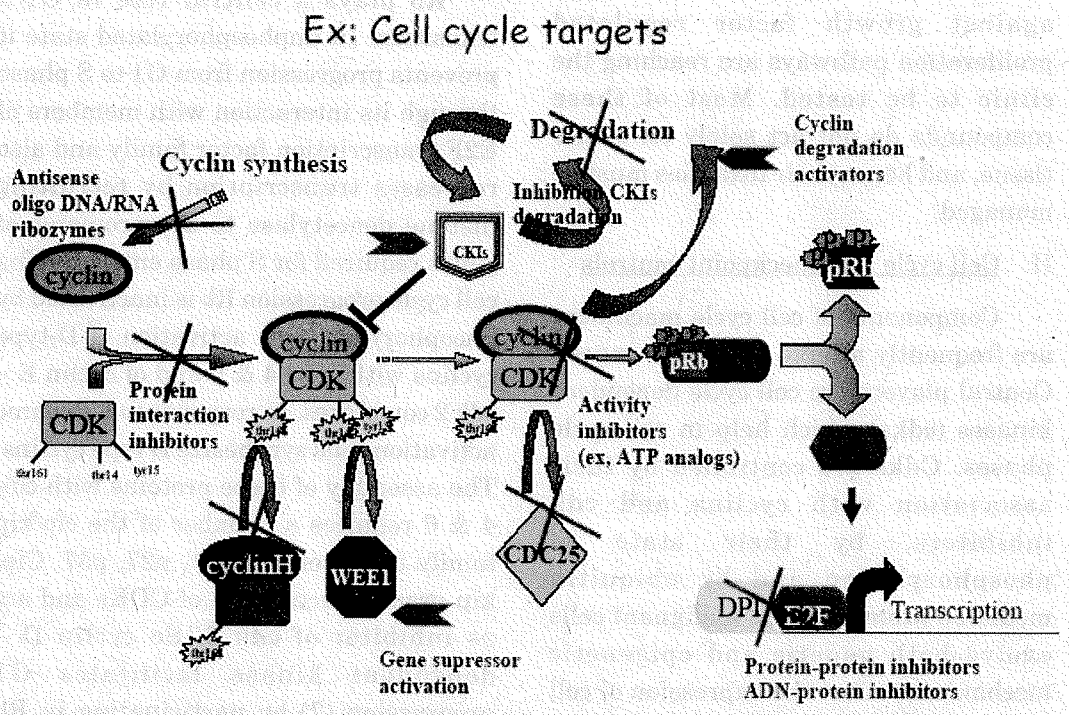
II.1. Drug targets – inhibition of cdk activity during the G1 phase.

Rb plays a central role in G1/S transition. In unphosphorylated state it prevents progression from G1 to S phase through its interaction with members of E2F transcription factor family and also represses transcription by recruiting histone deacetylase to the promoter of genes required for S phase entry. During cell cycle progression Rb is inactivated by phosphorylation by activation of D-type cyclins with cdks 4 & 6 and of cyclin E – cdk 2 complexes. In response to mitogenic activation cells synthesize D type cyclins. The assembly of these proteins with cdk 4 & 6 requires a member of the cip/kip family of proteins – p21, p27, p57. Cip/kip promote activation of CDKs and act as inhibitor of cdk 2. So cyclin D – dependent kinase facilitates G1 progression (7) by participating in Rb phosphorylation which relieves transcriptional repression by the Rb-E2F

complex sequestering cip/kip protein which facilitates the activation of cyclin E – cdk 2 (8). Cyclin E – cdk 2 – mediated Rb phosphorylation disrupts the binding of Rb to E2F, allowing E2F activation and transcription of genes necessary for S phase entry and progression. Rb is the primary target of cyclin D-dependent kinases. G1 progression is also regulated by INK4 family which acts as specific inhibitors of cdks 4 & 6. Most common alterations in tumors having wild type Rb is the inactivation of p16 INK4A by gene deletion, point mutation and transcriptional silencing by methylation (Figure 2). An inhibitor which blocks the ATP binding site of cdk4 maintains Rb-E2F as an active transcriptional repressor and promotes G1 arrest, but not in tumors lacking Rb?

The cdk inhibitors include several classes of drugs, all derived from microbial and plant sources. Of these flavopiridol and UCN-01 (7-hydroxystaurosporine) are in clinical

Figure 2



Flavopiridol: CDK inhibitor resulting in cell cycle arrest at G1/S and G2/M
Bryostatin-1: CDK2 inhibitor resulting in cell cycle arrest at G2

trials. They both bind to the ATP-binding pocket of the kinase and block cell cycle progression at G1 phase and change the levels and distribution of endogenous cdk inhibitors, independent of p53. The cdk inhibitor purvalanol B is more potent and shows high degree of selectivity for cdks. Short peptides are being investigated that block the interaction of cyclin A – cdk2 with substrates such as E2F1. They induce S phase arrest and cause abrupt apoptosis and are selective for transformed cells. Drugs that facilitate mitotic entry following DNA damage – G2 checkpoint abrogators therefore sensitize cells to chemotherapy and radiation and this sensitization is selective for p53 – deficient cells making them attractive as novel antineoplastics.

The development of drugs that target specific cellular pathways can improve efficacy, lower toxicity and lower costs – personalized medicine. Strategies designed to reduce cdk4 activity that would also cause cip/kip protein to redistribute into complexes with cyclin E and cdk 2 may be preferable to the use of drugs that simply block the ATP-binding site of cdk4. Such strategies include altering cdk4 stability, reducing cyclin D levels, replacing p16 INK4a expression using adenovirus vectors or reactivating methylated p16 INK4a. (Figure 2)

III. Angiogenesis

It is the process of *de novo* formation of vasculature that nourishes the growing tumor with oxygen and nutrients. Sprouting angiogenesis involves multiple linked and sequential steps that include endothelial cell proliferation, migration, invasion, survival and capillary tube formation, mediated by multiple factors. The strategy of antiangiogenesis therapy provides an alternate to controlling the tumor. All solid tumors show angiogenesis. The targeted vascular endothelial cells are normal, genetically stable cells and therefore less likely than tumor cells to become drug resistant. Thus antiangiogenic therapy must be endothelial cell-specific and must distinguish between tumor and normal vasculatures. Tumor vessels are poorly structured and immature i.e. have incomplete coating with periendothelial cells. Immature vessels depend on survival factor. There are certain cell surface proteins on angiogenic tumor vessels, for eg. VEGF receptors and $\alpha_V \beta_3$ and $\alpha_V \beta_5$ integrins are relatively weakly expressed in normal endothelium and might provide useful targets for inhibition of tumor vessel angiogenesis. Angiogenesis is activated by perturbation in oxygen homeostasis and hormones. Both pro and anti angiogenic factors co-exist within the same tissue and the process of angiogenesis is mediated by specific interaction between them, at multiple levels i.e. between protein, including ligand-receptor, extracellular matrix, and antiangiogenic factor interactions. Thus identifying peptide domains that are specifically involved in tumor angiogenesis i.e. isolation of peptides from phage display libraries that bind to proteins that are preferentially

expressed on tumor blood vessels, has proved helpful in uncovering receptor selective expression on tumor vasculature. A chimeric peptide containing

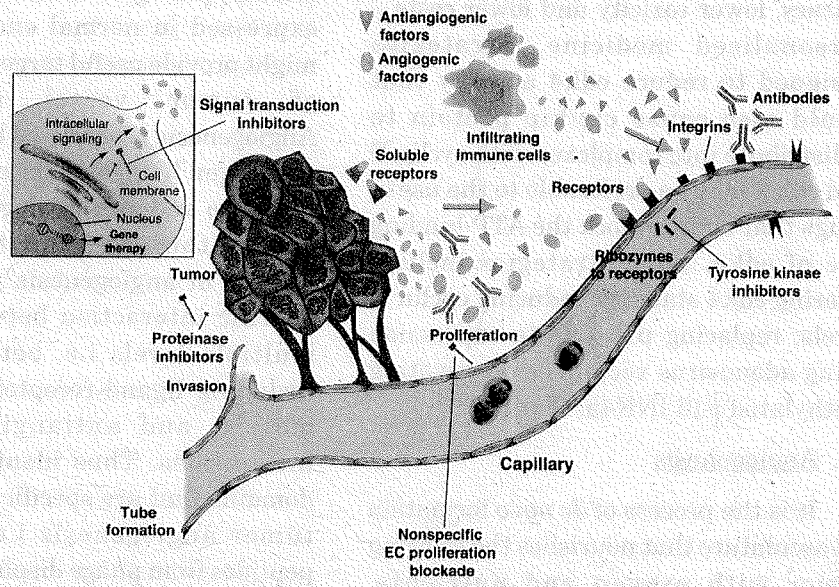
a tumor blood vessel homing motif and a pro-apoptotic peptide was selectively toxic to angiogenic endothelial cells and showed anticancer activity in mice (9).

III.1. Antagonizing proangiogenic activities

Angiogenic stimulus comprises of a series of steps which include local degradation of bone marrow, directional migration of the underlying endothelial cells, invasion of the surrounding stroma, endothelial cell proliferation, capillary tube morphogenesis, coalescence of capillaries into large vessels, vascular pruning and acquisition of a periendothelial cell coating. Thus, tumor neovascularization could be inhibited at each of these strategic junctions VEGF family i.e –A, -C, R1, R2 are upregulated in angiogenesis (Figure 3). VEGF plays a role in both vasculogenesis and angiogenesis and is an embryonic cardio vascular morphogen. It is tightly

Figure 3

Strategies to Inhibit Tumor Angiogenesis



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regulated during development. Different classes of VEGF antagonists include anti VEGF neutralizing antibodies, inhibitors of VEGF-R2 tyrosine kinase, soluble versions of VEGF-R1 & R2.

VEGF is strongly induced by hypoxia and hypoglycemia. It functions as a survival factor for immature blood vessels which become VEGF independent only upon their maturation and engagement with periendothelial cells. For eg. early regression of blood vessels on androgen ablation in prostate cancer is due to suppression of androgen-regulated VEGF production. VEGF has dual role as an angiogenic factor and a vascular survival factor. In vicinity of primary tumor, pro-angiogenic signals override anti angiogenic, resulting in growth, but in remote sites the balance tilts in other direction because pro-angiogenic factors have short half life, whereas antiangiogenic factors in circulation last longer creating a net inhibitory effect.

III.2. Antiangiogenic therapy

Angiostatin is an antiangiogenic protein, a 38 KD internal fragment of the serum protein plasminogen. Another protein endostatin is a terminal fragment of collagen XVII a component of blood vessel wall. Both proteins inhibit VEGF activity, are non toxic, non immunogenic and natural body proteins. Introduction of DNA that codes for antiangiogenic factor, circumvents the need for daily injection. Another strategy is that of inducing the release of antioangiogenic peptides from their endogenous precursor proteins, e.g. conversion of plasminogen to angiostatin using tissue specific plasminogen activator or streptokinase (10).

III.3. Modulation of endothelial cell-ECM interactions

The extracellular matrix (ECM) has a profound effect on the angiogenic phenotype through multiple interactions with endothelial cells and transduction of signals by cross-linking integrin receptors on endothelial cells. In addition MMP (matrix metallo proteases) appears to be specific for angiogenesis. Antiangiogenic therapeutic approaches target MMP activity (11). Integrin mediates adhesion of endothelial cells to ECM components. Antagonists of α integrin are in phase II clinical trials. α_v p_3 mAb (vitaxin) is in trials. The identification of receptors for newly discovered antiangiogenic proteins might open new routes for therapeutics with small molecules.

IV. Apoptosis

In cancer the goal is to trigger tumor selective cell death. Biology of neoplasia has expanded to incorporate not only lesions that cause dysregulation of growth but also those that lead to inefficient cell death. Apoptosis represents an efficient cellular suicide pathway. Molecular pathways whose end point is death/ apoptosis coincide with the goal of successful treatment. Expression of apoptotic modulators within a tumor appear to correlate with its sensitivity to traditional cancer therapies. A drug that activates apoptosis might achieve a

therapeutic index in several ways – (1) activate a death cascade via a drug target uniquely expressed in a cancer cell. (2) it might be delivered to the target tissue in a manner that is selective for the cancer cell. (3) most promising strategy is to exploit a pathway that is activated by oncogenes, in order to provoke apoptosis selectively in cancer cells. Oncoproteins can interact with apoptosis regulatory pathways. Thus overexpression of Myc sensitizes cells to a wide assortment of apoptotic trigger, probably reflecting the role of apoptosis in the intracellular immunity that prevents normal cells from persisting in the body once they acquire cancer causing genetic defects. Thus oncogenes can sensitize cells to pro apoptotic treatment (12).

Apoptotic cell death is triggered by intracellular cues such as DNA damage and osmotic stress and extracellular cues including GF withdrawal, matrix detachment and direct cytokine mediated killing. Two central pathways are involved in the process of apoptotic cell death, one involving the activation of caspase proteases and a second the mitochondrial pathway. Caspase activation is regulated by adaptor molecules (FADD) that promote or inhibit caspase activation (IAPs). A number of death pathways converge on caspase cascade. Some of these begin when a death ligand such as TNF or Fas L interacts with its cognate receptor. TNF-R or Fas (CD95) induces the trimerization of the receptors. These adaptor molecules then activate caspase-8.

The second, mitochondrial pathway to apoptosis is the likely province of the Bcl-2 family of proteins which contain both pro apoptotic factosr such as Bax, Bak, Bcl-XS and anti apoptotic factors such as Bcl-2, Bcl-XL. In response to apoptosis signals, pro-apoptotic Bcl-2 family members translocate to and alter the permeability of the mitochondrial membrane by forming channels, leading to changes in mitochondrial membrane potential, cytochrome C release and the production of reactive oxygen species. Antiapoptotic members of Bcl-2 family reside in the outer mitochondrial membrane and counter these effects. The mitochondria and caspase apoptotic pathways are intimately connected, leading to activation of caspase 9 and 3.

IV.1. Drug targets in apoptosis

1. Inhibition of Bcl-2 by antisense oligos, antisense mRNA, ribozyme constructs, peptides that mimic the domain of Bax which mediates binding to Bcl-2 would disrupt the interaction of Bcl-2 with Apaf-1, which suppresses caspase-9 activity.
2. Activating death pathway directly using soluble death ligands
3. NF-κB is a transcription factor composed of dimers of the NF-κB/ Rel family of proteins. Its activity is induced by a number of stimuli, including cytokines TNF-α, IL-1 and viral infection. It mediates a critical anti apoptotic signal, and so inhibition of NF-κB appears to lead to cell death. Targets of NF-κB

regulation include the IAPs, TRAF-1 & -2 which are thought to suppress caspase-8 activation. Multiple human tumors (Hodgkins) and an avian retrovirus have evolved mechanism for dysregulating the NF-κB pathway, suggesting that NF-κB participates in cellular transformation. Drug targets in NF-κB pathway include therapeutic inhibitors of NF-κB activation, IKK-2 inhibitor as IKK-2 is a regulator of IκB whose inhibition should block the disruption of the IκB / NF-κB complex, adenoviral mediated delivery of IκB α targeting NF-κB at the level of transcriptional activation. NF-κB activates transcription through the p300/CBP family of co-activators, so molecules that disrupt this complex could prove therapeutic.

4. P53 is the most commonly mutated gene in human cancer. It regulates cell cycle and apoptosis. Restoration of p53 pathway in p53-deficient cells is a promising approach for controlling tumors by gene transfer vectors, targeted delivery of transgenes – to enhance apoptosis only in tumors in which the p53-apoptotic pathway is otherwise intact.

5. P13K/AKT pathway is a potent mediator of cell survival signals. Extracellular survival signals delivered as soluble factors or through cell attachment can inhibit apoptosis by activating this pathway. PI3K acts on membrane phosphoionosides to generate PI 3, 4 bis PO4 and PIP3, which serve as foci for recruiting and activating a number of signaling proteins to the membrane. Among these proteins are AKT family. Overexpression of AKT can confer cell survival. AKT phosphorylates and inactivates a number of substrates that are connected with apoptosis such as caspase 9, BAD. AKT can also inhibit cytochrome C release and thus affect apoptosis at multiple points. PI3K/AKT pathway is attractive target for therapeutic intervention.

V. Anticancer drug targets - Oncogenes and tumor suppressor genes

Among the genetic damage seen in cancer are dominantly acting mutations that convert proto-oncogenes to oncogenes as well as recessive mutations that inactivate tumor suppressor genes. Majority of hereditary cancer syndromes are due to germ line mutations in tumor suppressor genes. Most human cancers harbour mutation that directly or indirectly inactivate the Rb protein e.g. mutations affecting genes such as p16/INK4A, cdk4 and cyclin D1 which lead to phosphorylation and functional inactive of pRB. Altered growth factor receptors, as well as oncogenic Ras, likely, also impinge on this pRB pathway. pRB is an important negative regulator of cell cycle progression and serves to integrate positively and negatively acting mitogenic signals. Loss of pRB leads to mitogen independence of cancer cells. Likewise, p53 is mutated in 50% of human cancers

and many tumors that retain wild type p53 allele harbour mutations in other genes that regulate p53. For eg. ARF negatively regulates p53. Downstream target of p53 is apoptosis inducer Bax, the function of which is antagonized by Bcl-2, which is overexpressed in a variety of tumors including nodular lymphomas.

The importance of p53 and pRB in carcinogenesis is underscored by the fact that a variety of unrelated DNA tumor viruses have independently evolved the means to inactivate both pRB and p53, for eg. the oncogenic HPV product E7 and E6 proteins that inactivate pRB and p53 respectively. Thus it may be possible to choose the most suitable drug target from a variety of structurally unrelated molecules that all function in the same signaling pathway. These pathways are not strictly linear but, rather, constitute molecular networks. For eg. loss of pRB leads to derepression of the E2F transcription factor family, which in turn, leads to the induction of ARF and subsequent activation of p53.

Thus the potential for molecular cross – talk between pathways has implications for the development of drugs that specifically target tumor cells while sparing normal cells (13). Not all the abnormalities in a cancer cell would need to be corrected to have a therapeutic efficacy. For e.g. restoring p53 function in p53 defective tumor cells is sufficient to induce cell death, to induce a cell-cycle block, or, in some to restore sensitivity to conventional chemotherapy. Most cancer causing mutations induce a loss, rather than gain of function. Loss of function of one molecule is often similar in consequence to a gain of function of another for eg. loss of cdk inhibitor p16/INK4A might be viewed as a gain of cdk 4 and cdk 6 (14). Genes are actually members of gene families eg. p53 homologs are p63 and p73 and these are rarely mutated in human cancers, but, they can substitute for p53 to induce apoptosis in p53 defective tumors. So, p53 function can be restored by using small molecules that can activate p73 or p63. Thus, one can exploit mutations that were selected because they were essential for the survival of the cancer cell.

VI. Molecular targets for nutrients involved with cancer prevention

Discovery of molecular pathways critical to carcinogenesis is revolutionizing the treatment and prevention of cancer. Compelling experimental epidemiology and clinical evidence indicates that many cancers are preventable, especially because diet and nutrition are key factors in the modulation of cancer risk. The road to nutritional intervention in cancer prevention has led to successful trials as well as trials that did not reach their intended endpoints (15). Nutrition is intimately involved in cancer prevention and use of food stuffs represents a cost effective and noninvasive strategy for reducing cancer risk. Dietary nutrients can influence cancer risk by inhibiting or enhancing carcinogenesis through diverse mechanisms and molecular interactions. Transforming nutrition and cancer

research from an observational to a molecular approach offers potential of identifying individuals who will and will not benefit from dietary intervention strategies. Genetic polymorphisms can also influence the dynamics between nutrients and molecular targets.

We need to embody new and innovative preclinical and clinical approaches to nutrition and genetics. Numerous dietary components such as essential nutrients, phyto or allelo chemicals in plants, carotenoids, flavonoids, indoles, isothiocyanates, etc. can potentially alter genetic and epigenetic events and gene-regulated metabolic pathways through interactions with specific molecular targets. The molecular targets may be individual genes, molecules that result from gene expression or are affected by gene expression. The processes influenced by nutrients are differentiation, cell cycle, cell signaling, apoptosis, carcinogen metabolism. The areas of interest are (1) characterizing molecular events that govern, the ability of specific nutrients to alter cell cycle checkpoints, (2) credentialing of target receptors for cancer prevention that are modified by dietary constituents (3) methylation patterns that are influenced by dietary manipulations that influence gene expression and cellular phenotypes and (4) use of natural genetic variations to elucidate how nutrient exposures are linked to phenotype (5) signaling pathways that regulate cancer growth, development, differentiation and apoptosis as regulated by dietary components. Vitamin D, calcium, folate, genistain, selenium, reservatrol, curcumin are being investigated in chemoprevention trials (16). (Table 2) lists some of the nutrients that modify cancer risk.

Table 2

Partial list of Nutrients that may modify cancer risk

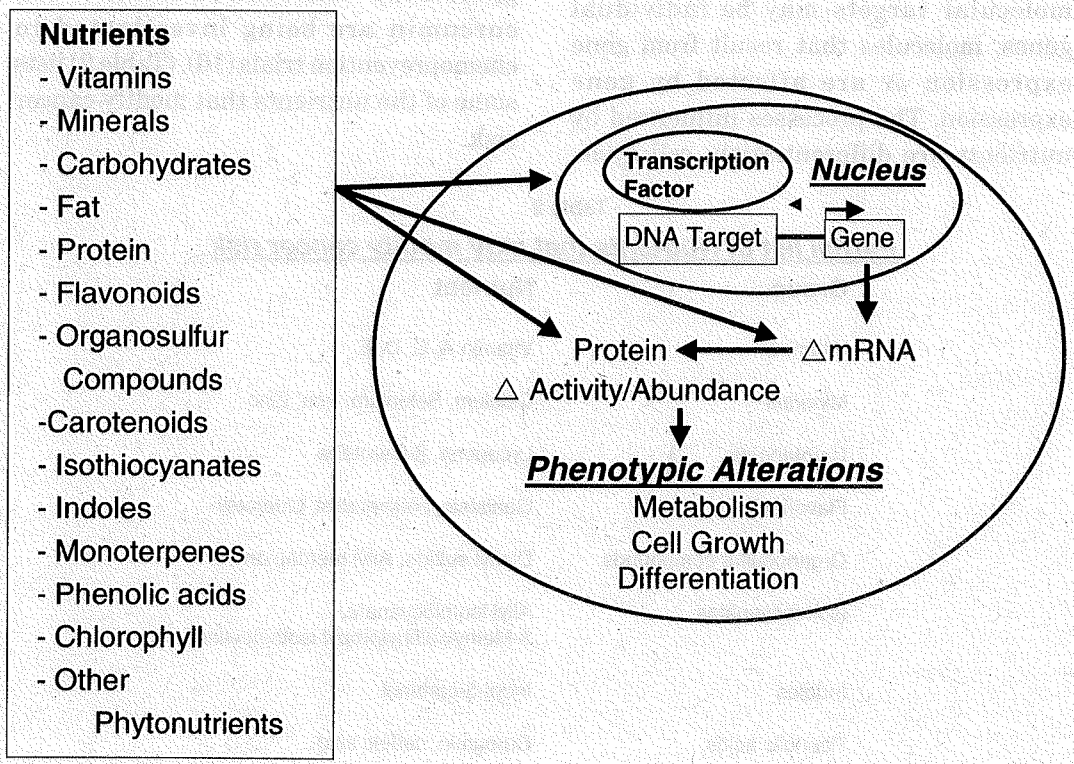
Group	Nutrient
Vitamins	Vitamin A, C, D, E
Minerals	Calcium, Selenium, Iron, Zinc
Carotenoids	Lycopene, β-Carotene
Flavonoids	Genistein, Resveratrol, Quercetin
Organosulfur compounds	Diallyl sulfide, Allyl mercaptan
Isothiocyanates	Allyl isothiocyanate, 3-Methylsulfinylpropyl isothiocyanate
Indoles	Indol-3-carbinol
Phenolic acids	Curcumin, caffeic acid

VI.1. Specific nutrients and their molecular targets

Nutrients act within numerous biochemical and molecular cascades involving nuclear and cytoplasmic events that regulate the amount and activity of specific proteins (Figure 4), which in turn influence cell proliferation, apoptosis and differentiation. Vitamin D and its metabolites act through a variety of molecular targets to inhibit carcinogenesis. Vitamin D binds to EGF receptor, reduces availability of EGF, with subsequent inhibition of growth and increased differentiation in normal and malignant cells. Vitamin D receptor binds to DNA adjacent to the jun-fos gene complex, inactivating jun, which is an activating gene for transcription. This results in repression of normal transcription and finally inhibition of cell proliferation. It also downregulates c-myc. Folate functions as a coenzyme in one-carbon transfer reactions in the metabolism of nucleic and amino acids. Folate deficiency results in altered DNA methylation, disruption of DNA integrity, and disruption of DNA repair and thus

Figure 4

Selected nutrients as regulators of gene expression



leads to increased risk for carcinogenesis especially of colorectal cancer. Cancer cells often show global hypomethylation which promotes genetic instability due to overexpression of oncogenes. It is also suggested that hypomethylation and DNA strand breaks resulting from folate deficiency might enhance the incorporation of tumorigenic viruses such as HPV into human DNA. Molecular targets for selenium have supported that besides its antioxidant property selenium has diverse biological functions for e.g. inhibition of proliferation, enhancement of immune response, alteration of metabolism of carcinogens and induction of apoptosis. Selenium plays a role in the thioredoxin system. The activity of thioredoxin reductase, a selenoenzyme has been linked to NF-κβ activation. NF-κβ is an inducible oncogenic nuclear transcription factor that responds to the redox state of the cells and has pivotal role in inducing genes involved in a number of physiological processes, including those associated with cytokines, GFs, adhesion molecules and immuno receptors. Selenium availability can also alter DNA methylation. Essential and nonessential nutrients cannot work in isolation but rather work together. Several factors might account for variability in response to dietary selenium. Vitamin C has been reported to reduce the effectiveness of selenium against chemically induced colon cancer, but selenium has been shown to enhance the ability of garlic to inhibit chemically induced mammary cancer in animals.

Certain classes of phytonutrients such as flavonoids, carotenoids, organosulfur compounds, terpenes and isothiocyanates have a range of molecular targets. Genistein a phytoestrogen present in soyabean can bind to estrogen receptor and exhibit estrogen like biological activity. It decreases the risk for certain hormone related cancers such as breast, endometrial and prostate. Genistein scavenges free radicals and is an antioxidant. It induces cell cycle arrest, inhibits PTK inhibitor, angiogenesis and metastasis and stimulates apoptosis by inhibiting antiapoptotic transcription factor NF-κβ.

Similarly resveratrol found in grapes and red wine is a good antioxidant. It inhibits ornithine decarboxylase a key enzyme which is enhanced in cancer cell proliferation. It inhibits Cox-2 activity through inhibition of PKC signal transduction pathways, influences the expression of Bax and p21 genes involved in the regulation of cell proliferation and apoptosis. It induces apoptosis through activation of p53 dependent transcription and caspase activation, and inhibition of NF-κβ.

Genetic differences also play a role in the ability of individuals to withstand exposure to exogenous carcinogens or to inhibit initiation, promotion or proliferation in carcinogenesis (17). Prevalence of polymorphisms in genes coding for activation of enzymes of cytochrome P450, detoxification enzymes glutathione-s-transferase may account for inter individual variation in cancer susceptibility and cancer prevention.

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