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Review Article

A REVIEW ON COMPREHENSIVE CANCER PATHWAYS

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ABSTRACT

Human life is constantly threatened by many diseases such as cancer. Cancer is the second leading cause of death worldwide. It is not a disease only of cells, but it is a disease of various systems and the components that interact at both molecular and cellular level to lead to initiation and progression of the disease. Since the network is typically complex, with multiple connections between the pathways and important feedback loops, it is crucial to represent it in the form of computational model that can be used for a rigorous analysis. Drugs are essential for the prevention and treatment of disease. To meet the challenges of ideal drugs, fanciful method of drug development is demanding. The process of drug development is challenging, time consuming, expensive, and requires consideration of many aspects. To fulfil these challenges, several multidisciplinary approaches are required for the process of drug development; collectively these approaches would form the basis of rational drug design. A comprehensive study of the cancer pathways were performed on a large number cancer types.This study oriented towards the relation between different cancer and apoptosis pathway and identify critical points in cancer signaling. A complete network analysis of cancer pathways in the cell helps to identify important proteins as co-targets, targeting which could encounter the emergence of cell survival. A connectivity map of several cancer pathways has been plotted along with apoptosis pathway. Finally, we concluded that the cancer pathways are proceeding by blocking apoptosis either through PKB/Akt or Casp9. Understanding of the complete cancer metabolic pathways and interacting networks provides an excellent model for systems biology development and analysis. The purpose of this review is to highlight examples of progress in cancer areas, indicate where knowledge is scarce and point out fertile grounds for future investigation.

Keywords: Cancer, Apoptosis, Connectivity map and Systems biology.

INTRODUCTION

The availability of complete genome sequences of several organisms has led to a paradigm shift from the study of individual proteins to the study of proteins interacting in networks in the cell. Unlike traditional biology, systems biology involves more holistic approach, which will capture and reveal elaborate webs of molecular interconnections. Diseases like cancer, involve a large number and variety of elements that interact via complex networks. Therefore, simply knocking out one target molecule in a biochemical pathway is not sufficient for treating a disease like cancer, because the cells often find alternative molecular routes to escape the blockage.

Cancer is the second leading cause of death worldwide. Cancer remains a fundamental burden to public health despite substantial efforts aimed at developing effective chemotherapeutics and significant advances in chemotherapeutic regimens. It is not a disease only of cells, but it is a disease of various systems and the components that interact at both molecular and cellular level to lead to initiation and progression of the disease. Since the network is typically complex, with multiple connections between the pathways and important feedback loops, it is crucial to represent it in the form of computational model that can be used for a rigorous analysis. During tumor development, cancer cells modify their metabolism to meet the requirements of cellular proliferation, thus facilitating the uptake and conversion of nutrients into biomass [1].

The major challenge in anti-cancer drug design is to selectively target cancer cells with high specificity. Research into treating malignancies by targeting altered metabolism in cancer cells is supported by computational approaches, which can take a leading role in identifying candidate targets for anti-cancer therapy as well as assist in the discovery and optimization of anti-cancer agents.

Targeting a broader range of related biological structures should result in compounds that have common structural and functional properties, and common mechanism of action, ultimately creating the potential for the application of a therapeutic to multiple diseases by targeting common pathways implicated in pathogenesis [2].Drug discovery is a challenging process and historically, has been long-drawn as well, with a significant trial - and- error element. In addition to having many uncertain phases, drug discovery is also an extremely laborious and expensive process, requiring millions of dollars and about 12- 15 years for a drug to reach the market from its initial discovery stage.

Systems are composed of individual elements or parts that interact in various ways. In general, the behavior of a `system is quite different from merely the sum of the interactions of its various parts. Systems biology signals a departure from the now common view in drug discovery of 'one target, one drug paradigm shift. Traditional cancer chemotherapy agents designed to block cell division are toxic to healthy cells as well as to cancer cells. Targeting specific metabolic pathways to stop cancer growth is potentially less toxic to normal cells and can improve tolerability considerably. Thus, anticancer drug discovery has shifted from the traditional empiric random screening approach to a more rational and mechanistic, target-based approach whereby specific abnormalities in cell functioning are modulated in a classical drug (ligand)-receptor fashion.

We start with a discussion of different types of cancers including breast cancer, prostate cancer, bladder cancer etc. and apoptosis.A dedicated section highlights a connectivity map, which will depict the linkage between the pathways and that could be the most promising strategies to develop anti-cancer therapeutics. We conclude with a brief summary of the most interesting strategies identified and with an outlook on future directions in anti-cancer drug development.Following sections describes various genes involving in different cancers.

Human Epidermal Growth Factor Receptor 2 (HER2), also known as ErbB2, c-erbB2 orHER2/neu, is a 185 kDa protein (p185) with an intracellular tyrosine kinase domain and an extracelluar ligand binding domain. In humans, HER family includes four structurallyrelated members, HER1 (ErbB1, also known as EGFR), HER2 (ErbB2), HER3 (ErbB3) and HER4 (ErbB4). Although HER2 is the only receptor which has no identified ligand, it is thepreferred partner to form heterodimer with other HER members. HER2 involvedheterodimerization is the most potent signal transduction pathway among all dimmersformed by the HER family [3]. HER2 plays important roles in cell growth, survival, anddifferentiation in a complex manner. The major signaling pathways mediated by HER2involve mitogen-activated protein kinase (MAPK) pathway and phosphatidylinositol 3kinase (PI3K) pathway. As a key gene for cell survival, HER2 gene amplification andprotein overexpression lead to malignant transformation [4]. It directly associates with poor clinical outcomes in breast, ovarian, gastric, prostate and other cancers.

Point mutations, deletions, and rearrangements in the tumor suppressor gene p53 found on chromosome 17p have been detected in human colon, lung, esophagus, breast, liver, brain, and hematopoietic tissue cancers[5]. The wild type p53 protein has been shown to be involved in the regulation of cellular growth [6, 7]and its expression suppresses the growth of transformed cells [8]. Recent evidence has shown that it is a DNA- binding protein [9]. Cancers of the lips, tongue, floor of mouth, palate, gingiva, buccal mucosa, and oropharynx account for 3% of all newly diagnosed cancers in the United States. The most common type of oral cancer is squamous cell carcinoma (SCC), which is found in approximately nine of ten oral malignancies. Epidemiologic studies have shown that age and tobacco and alcohol use are the predominant risk factors in the development of oral cancer[10]. Exposure to ultraviolet radiation is an additional risk factor in the development of SCC of the lip.

The molecular basis of oral cancers is incompletely understood. The protooncogenes c-myc, N-ras, and Ki- ras have been reported to be amplified in a large percentage of oral cancers, although an association between amplification and the size or degree of differentiation of the tumors has not been definitively shown[11,12,13]. It was[14] showed increased levels of c-Ha-ras, c-Ki-ras, and c-myc RNA expression over c-oncogene levels in normal tissues in nine fresh oral SCC specimens. Clinical parameters such as sex, site, stage, degree of differentiation, and outcome were not correlated with c-Ha-ras and c-Ki-ras expression levels, although increased c-myc levels were correlated with higher TNM stages[15].

Breast cancer is the most common malignancy among women, and the second leading cause of cancer deaths in the United States[16]. The 5-year survival rate for women diagnosed with metastatic breast cancer is only about 27.4% [17]. Although adjuvant chemotherapy, as well as radiation, hormonal, and targeted therapy, have all been used to treat different stages of breast cancer, chemotherapy continues to be the major therapeutic option for patients with metastatic breast cancer [18]. While many cytotoxic drugs (including doxorubicin, vinorelbine, gemcitabine, *nab*-paclitaxel, pemetrexed, platinum salts, etoposide, and irinotecan) have been developed for the treatment of metastatic breast cancer, the response rates for these chemotherapeutic agents are usually poor, and the frequency at which patients develop drug-resistance remains high[19]. One major problem with chemotherapy for the treatment of cancer is the lack of selective toxicity, which results in a narrow therapeutic index.

Gene amplification and/or overexpression of some oncogenes have been implicated in breast cancers. HER2/neu (also known as ErbB2) is among the most characterized oncogenes linked with poor prognosis in breast cancer[20]. Overexpression of HER2/ neu is found in 30% of human breast cancers and correlates with more aggressive tumors and more resistance to cancer chemotherapy [21]. Anincrease in HER2/neu expression also enhances malignant phenotypes of cancer cells, including those with metastatic potential [22,23,24].The ErbB2/3 heterodimer efficiently activates the phosphatidylinositol 3-kinase (PI3K)/AKT/PTEN pathway and the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, which are essential in cellular survival, by phosphorylating and inactivating growth-inhibitory and proapoptotic proteins. Aberrant expression of ErbB receptors triggers the activation of multiple downstream signal transduction pathways and plays a key role in inducing increased cell proliferation and differentiation, decreasing apoptosis, and enhancing tumor cell motility and angiogenesis.

Mutations in the breast and ovarian cancer–susceptibility genes BRCA1[25] and BRCA2[26, 27]are found in a high proportion of multiple-case families with breast cancer, especially if they also include one or more case patients with ovarian cancer [28].

Colorectal cancer accounts for 10% to 15% of all cancers and is the second leading cause of cancer deaths in western countries. Approximately half of all patients develop metastatic disease[29]. The epidermal growth factor receptor (EGFR), which participates in signaling pathways that are deregulated in cancer cells, commonly appears on colorectal-cancer cells. Palliative chemotherapy is more effective than the best supportive care at prolonging survival and improving quality of life [30]. Until recently, the antimetabolite fluorouracil (FU), which has been available for over 40 years, and leucovorin (LV) modulation were the standard of care, despite having no major impact on survival.

The most important step leading to the diagnosis of a hereditary cancer syndrome is the compilation of a thorough family history of cancer[31,32]. A patient and his or her key relatives, working either alone or with a trained nurse or genetic counselor, can compile such a detailed family history. The focus should be on identifying cancer of all types and sites; the family member's age at the onset of cancer; any pattern of multiple primary cancers; any association with phenotypic features that may be related to cancer, such as colonic adenomas; and documentation of pathological findings whenever possible. This information will frequently identify a hereditary colorectal cancer syndrome in the family, should it exist. Molecular genetic testing may then provide verification of the diagnosis, when a germ-line mutation is present in the family [33,34,]. The primary care physician may wish to refer the patient to a hereditary-cancer specialist and genetic counselor for further evaluation should there be any remaining question about the disorder's clinical or molecular genetic diagnosis and the need for targeted surveillance and management.

Expression of vascular endothelial growth factor (VEGF) was 3-fold higher and that of platelet-derived endothelial cell growth factor was 40-fold higher in tumors compared to normal bladder. However, the factors were differentially expressed in different stages of the cancer. A normal urothelial cell transforms to a malignant cell and then metastasizes is a complex process that involves the interaction of many different genes, proteins, and other molecules. Loss of tumor suppressor gene function or induction of oncogenes can lead to unregulated cell growth and proliferation. Environmental chemicals are thought to play a significant role in bladder cancer initiation. Carcinogens derived from occupational exposures, cigarette smoking, and inflammatory conditions associated with long-term indwelling foley catheters and schistosomal infections are important factors in initiating bladder cancer. Furthermore, prior pelvic irradiation and cyclophosphamide exposure also appear to be important risk factors, possibly from direct mutagenesis.All of these conditions may lead to genetic changes, which irreversibly convert a normal urothelial cell to one with the malignant phenotype. Normal cells express the Rb protein, while mutations or gene deletions, which often result in lack of protein expression, may be identified by the lack of Rb expression. Loss of heterozygosity at the Rb locus associates strongly with the absence of Rb protein expression. Rb gene mutations are seen in approximately 30% of bladder cancers[35].

Prostate cancer is the most common cancer in men resulting in over 2,32,090 new cases and 30,350 deaths annually[36]. Prostate cancer is a leading cause of cancer-related death in males. Loss of function mutations in tumor suppressor genes or activating mutations in oncogenes have been shown to dysregulate signal transduction pathways leading from growth factors (such as EGF 3) and their cognate receptor tyrosine kinases to PI3K, which catalyzes the conversion of phosphatidylinositol 4-phosphate, and phosphatidylinositol 4,5-biphosphate to phosphatidylinositol 3,4-biphosphate and phosphatidylinositol 3,4,5-triphosphate, respectively [reviewed in Ref. 38]. These products are allosteric activators of phosphatidylinositol-dependent kinase 1, which phosphorylates and activates AKT (protein kinase B). Targets of AKT include BAD, an inhibitor of apoptosis, and FRAP, an activator of p70 s6k, which is required for ribosomal biogenesis and cell cycle progression.These findings have delineated mechanisms by which the PI3K/AKT pathway promotes cell proliferation and inhibits cell death. This pathway is negatively regulated by PTEN, which dephosphorylates phosphatidylinositol 3,4- biphosphate and phosphatidylinositol 3,4,5-triphosphate [37].

Activation of PI3K and the generation of phosphatidylinositol 3,4-bisphosphate and phosphatidylinositol 3,4,5-trisphosphate in vivo are necessary for the activation of Akt/PKB, a downstream mediator of PI3K signaling, through phosphorylation of Thr-308 and Ser-473 by PDK1 and PDK2/integrinlinked kinase [38]. In numerous cell

types, it has been shown that Akt/PKB induces survival and suppresses apoptosis induced by a variety of stimuli, including growth factor withdrawal and loss of cell adhesion. The mechanisms by which Akt/PKB regulates cell survival involve the phosphorylation and inactivation of the apoptotic mediators BAD [39],caspase-9 [40],FKHRL1 [41], and IKK- [42,43]. Akt/PKB is also involved in regulating cell proliferation [44,45].

Reactive oxygen species (ROS) such as superoxide (O_2^o) or its breakdown product hydrogen peroxide (H_2O_2) have been implicated in the development of several diseases such as diabetes, heart disease, mitochondrial disease, various neurodegenerative diseases and cancer. ROS play a major role in tumor initiation induced by a variety of agents both in animal models of disease and also in humans[46,47, 48,].

The reoxygenation of blood and the reperfusion of hypoxictissue can increase the concentrations of free oxygen radicals[49]. ROS produced by hypoxia/reoxygenation have damaging effects in cells, cause tissueinjury, but also play a crucial role in vascular angiogenesis[50]. Tumors rapidly outgrow their blood supply leading to glucose deprivation and hypoxia. Glucose deprivation depletes intracellularpyruvate, thus preventing the decomposition of endogenous oxygen radicals [51]. In response to hypoxic conditions, breast carcinomas stimulate blood vessel development (angiogenesis). The blood flow within these new vessels is often chaotic and causes periods of hypoxia followed by reperfusion, which causes release of ROS[52]. ROS can increase the production of the angiogenic factors IL-8 (Interleukin-8) and VEGF (Vascular Endothelial Growth Factor). Additionally, ROS promote the secretion of the matrix metalloprotease MMP-1 by tumorcells, which promotes vesselgrowth within the tumor microenvironment. Blood vessel growth within the tumor increases the risk of blood-borne metastases. Another way by which oxidative stress increases blood supply is by triggering vasodilatation. ROS can activate heme oxygenase-1, which creates carbon monoxide, or induce iNOS (inducible Nitric OxideSynthase) whose product is nitric oxide (oNO). Both carbon monoxide and nitric oxide are vasodilatators [53].

In most cell types, NF-κB dimers are sequestered in the cytoplasm by IκB proteins. NF-κB must translocate to the nucleus to interact with κB-sites located in the regulatory regions of target genes. Nuclear translocation of NF-κB can be driven by two distinct signaling pathways, the classical activation pathway and the alternative activation pathway[54]. Proinflammatory cytokines, some members of the tumor necrosis factor superfamily(TNFSF) and pathogen-associated molecular patterns (PAMP) bind specific receptors that belong to the tumor necrosis factor receptor superfamily (TNFRSF) or Toll-like receptor (TLR)/interleukin (IL)-1R superfamily, leading to activation of the IκB kinase (IKK) complex, which consists of IKK α, IKK β and NEMO (NF- κB essential modulator, also known as IKKγ). In the classical activation pathway, activated IKK β phosphorylates specific serine residues of IκB in a NEMO-dependent manner,which results in polyubiquitination and subsequent degradation of I κB by 26S proteasomes. Then, released NF-κB, primarily the p50-RelA heterodimer, translocates to the nucleus, binds the κBsite and activates gene transcription. The alternative activation pathway is IKK α-dependent and IKK β and NEMO-independent. When LTβ R, BAFF or CD40, members of the TNFRSF, are stimulated by appropriate ligands, the IKKα homodimer is activated and phosphorylates specific serine residues in the C-terminal half of p100, which forms heterodimers with RelB. This phosphorylation leads to polyubiquitination-dependent degradation of the C-terminal half of p100 to generate p52-RelB heterodimers, which then translocate to the nucleus and activates the target gene[55].

Beta-catenin (or β-catenin) is a dual function protein, regulating the coordination of cell–cell adhesion and gene transcription. Mutations and overexpression of β-catenin are associated with many cancers, including hepatocellular carcinoma, colorectal carcinoma, lung cancer, malignant breast tumors, ovarian and endometrial cancer[56,57].

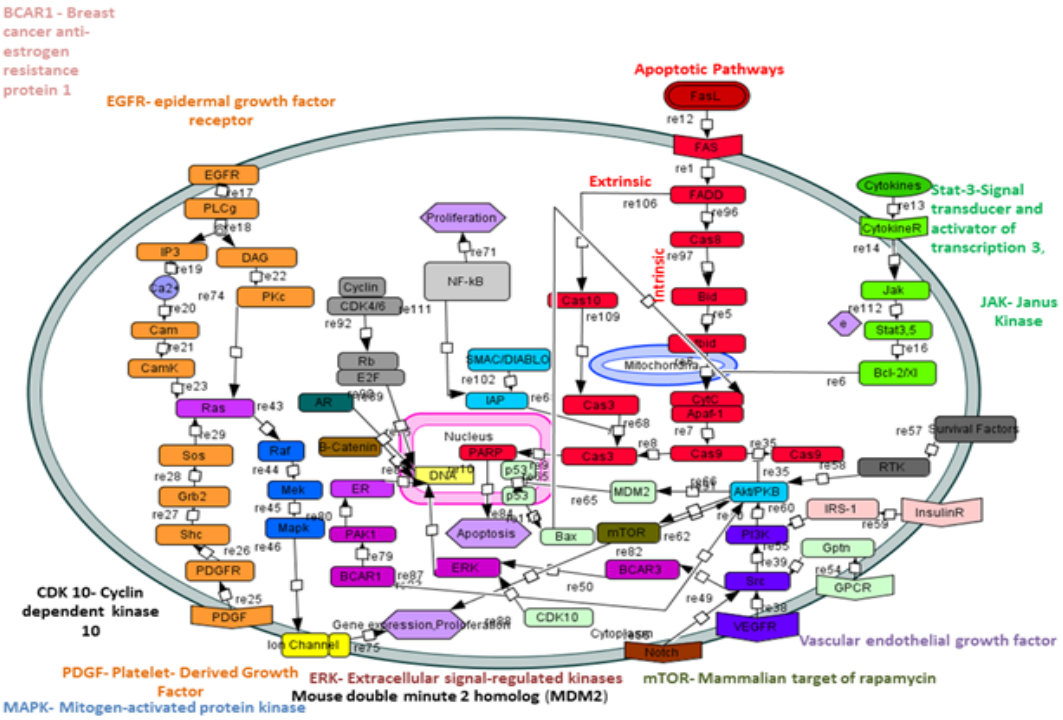

Fig. 1: A Comprehensive Cancer Pathway

Figure.1 depicted the comprehensive pathway of cancer. Different cancer pathways are plotted in a single view in connection with apoptosis. It was clear from the picture that the Cytokines cause cancer through Jak, Stat pathway by inhibiting the release of Cytochrome c from mitochondria.

GPCR, VEGFR, BCAR, survival factors etc. are interconnected. These pathways were promoting the survival or proliferation through the activation of Akt/PKB, which in turn blocks the activation of effector protein Casp9. NFkB lead to cancer via the inhibition of Casp3 protein, which was also an effector apoptosis promoter gene.

Table 1: List of different genes involved in different types of cancer

Endo metrial cancer	Colo-rectal Cancer	Small cell lung cancer	Basal Cell carcinoma	Bladder cancer	Chronic Myeloid Leukomia	Non-small cell lung cancer	Pan-creatic cancer	Thyroid cancer
FGFRs	DOC	cIAPs	SHH	FGFRs	BCR-ABL	PKC	TGFalpha	TRK
Ras	Casp9	Bcl-xL	Cholesterol	Ras	CBL	BAD	EGF	BRAF
Raf	Appl	TRAFs	PTCH1	Raf	CRK	Casp9	EGFR	BRAF
Mek	Kras	Cox2	SMO	Mek	Grb2	PKC	Jak1	Mek
Erk	Raf	iNOS	CoS2	Erk	Shp2	BAD	Stat3	Erk
MSK1	Mek	Max	GLI	MSK1	PI3K	Casp9	Stat1	DNA
C-Myc	Erk	Myc	DNA	C-Myc	PIP3	PI3K	VEGF	Ras
DNA	c-Fos	CKS1	SMP	DNA	PKB/Akt	PIP3	PI3K	BRAF
CyclinD1	DNA	Skp2	HIP1	CyclinD1	Bad	PKB/Akt	PIP3	Mek
CDK4	CyclinD1	p27Kip1	GLI1	CDK4	Bcl-xL	PDK1	PKB/Akt	Erk
Rb	Kras	CDK2	PTCH1,2	Rb	p53	EM4ALK	NFkB	RXR
E2F	RalGDS	CyclinE	Wnt	E2F	Sos	PI3K	RacGEF	PPARg
Shc	Rac/Rho		Frizzled	Shc	Ras		Rac	Ligands
Sos	JNK		DVP	Sos	Raf		Kras	ECAD
VEGF	PI3K		GSK-3BETA	VEGF	Mek		Raf	TCF/LEF
MMPs	PKB/Akt		Beta-catenin	Shc	Erk		Mek	Cyclin C
	GSR3B			MMPs			JNK	Cyclin D1
	Betacatenin						TGFbeta	
	TCF/LEF						TGFbetaR1	

From the table.1, it was clear that many genes are involved in many cancers and also same gene is involved in many cancers. So targeting one single protein was not sufficient for treating the disease. A comprehensive connectivity diagram of all possible cancer pathways open a new window for finding novel therapeutic drugs, where multiple ways were processing the disease.

CONCLUSION

Systems are composed of individual elements or parts that interact in various ways.Systems biology signals a departure from the 'single target, one drug' paradigm. Targeting a broader range of related biological structures should result in compounds that have common structural and functional properties, and common mechanisms of action, ultimately creating the potential for the application of a therapeutic to multiple diseases by targeting common pathwaysimplicated to the disease. Systems biology also has the potential to address several vital issues in drug discovery such as possible side effects and causes of drug toxicity. Hundreds of proteins might be involved in signaling processes that ensure the proper functioning of a cell. Many of the processes involved in tumor growth, progression and metastasis are mediated by signaling of various pathways. Taken together of these cancer pathways with apoptosis have provided a greater understanding of the role of certain mediators of cancer development by blocking apoptosis. When drugs are targeted to attack particular proteins, alternate pathways predominantly driven by isozymes may often be ignored, if a global system-level view is not considered. Here, different cancer pathways has been plotted in a single file along with the apoptosis pathway, which showed that each one are correlated and cause cancer by blocking apoptosis pathway either through promotion of Bcl-xL or suppression of Akt/PKB protein. If such a signaling network is altered, a caner phenotype could be generated.Targeting multiple points in a metabolic pathway can also be a useful strategy in drug design, perhaps explaining why combination therapy is popular. Understanding the complex systems involved in cancer will make it possible to develop smarter therapeutic strategies, for example, by disrupting two or three intersections in a biochemical pathway at the same time. Often, adverse drug reactions might emerge on account of the binding of the drug to proteins other than the intended targets. By considering larger systems and accounting for such possibilities, it is possible that such problems may be identified. These approaches could lead to significant advances in the treatment of cancer and help in transforming traditional reductionism- based approaches into systems level approaches for drug discovery.

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