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Normal Cell Signaling Pathways Maintains 'Stemness' in Stem Cells But Aberrant Pathways Causes Cells to Transform Into Cancer Cells – A Review

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Abstract: **Cell signaling pathways play significant role in cell fate in naive stem cells and mature cells. They maintain homeostasis by signaling for cell multiplication leading to genesis of new cells either from existing cells or from stem cells. The constant pool of functional cell type of particular tissue/organ is maintained at all the times. Constituents of cell signaling are extra-cellular cell surface markers and intra-cellular proteins. Signaling pathways are critical in stem cells to maintain 'stemness' leading to hallmark properties like self-renewal and plasticity.**

Abnormal signaling proteins may lead to loss of routine function of the pathway which may subsequently results into disastrous consequences ranging from different disorders to forming malignant neoplasm. The reasons for abnormal protein could be mutation in the gene which codes it or heredity. Stem cells and cancer cells share many properties like self-renewal & high level of acclimatization into the microenvironment they are in. Only difference being stem cells are highly regulated while cancer cells are not in terms of control over cell multiplication. Literature reveals involvement of abnormal signalling proteins in transformed cells.

It is imperative to study different pathways in cancer cells to device possible therapeutic strategy to reverse abnormal signaling protein or pathway with the help of normal stem cell pathway. The underlying mechanism behind this approach is cell to cell contact leading to possible repair of malignant cell type.

The present review article deals with seven key cell signaling pathways possibly involved in transforming cells intomalignant cell type. The article compares normal pathway with aberrant pathway in cancer cells. This concise approach is helpful in identifying the potential pathway signaling protein to be targeted for therapeutic application.

Keywords: **Stem Cells, Wnt Signaling Pathway, Neoplasm, MAP Kinase Signaling Pathways, Cancer.**

I. INTRODUCTION

Cancer is group of diseases involving malignant neoplasm, cells involved in forming tumor loses control on cell division by either external or internal factors. One of the hallmark properties observed in cancer cells is the aberrant cell signaling pathways which leads them to divide in uncontrolled manner. The external factors which cause cells to become uncontrolled include various chemicals, infections, radiation, and physical agents like asbestos etc. While internal factors include diet and exercise, hormonal imbalance, inheritance and stress. However at cellular level changes to normal cells to become cancerous include epigenetic alterations, aberrant behaviour of DNA repair machinery, loss of cell cycle regulation, modification at genetic level and alterations in signal transduction pathways.

Cells in multicellular organisms communicate with each other by mechanism called signaling or signal transduction. Communication by signal transduction is also evident in unicellular organisms. For example in human gastrointestinal

tract, bacteria tend to communicate with each other via signaling molecules, they also communicate with host epithelial cells and with cells involved in immunity [1]. This process is mediated by proteins which act as messengers between cells. Signaling molecules passes signals for various purposes like to start cell division, cell metabolism, apoptosis, immune response to infection, tissue/organ homeostasis by switching the expression of particular gene either 'on' or 'off'. These signaling molecules are sensed by cells through cell surface receptors. These signaling molecules together with cell surface receptors and entire intracellular signaling cascade forms a particular signaling pathway. The ability of cells to sense a particular signaling molecule in the microenvironment thereby initiating proper intracellular cascade is the basis of normal development, tissue repair, homeostasis and immunity. The error in this information processing and response leads to many diseases such as cancer, diabetes and autoimmune disorders.

Cells tend to communicate in three different ways, namely endocrine, paracrine and autocrine signaling. Endocrine signaling is characterized by communication between cells in distant regions. In paracrine signaling, both communicating cells reside quite closer. Both above signaling mechanisms can be acquired by cancer cells in advance stages helping them to invade surrounding tissues first and then moving out to distant sites there by metastasizing entire cancer. However, autocrine signaling is the peculiar mechanism acquired by cancer cells there by conferring them autonomy of cell division. Autocrine signaling in cancer cells not only allows them to synthesize their own growth factors but also cells get stimulated by bearing cell surface receptors for these growth factors.

It is estimated that somatic cells of human body undergoes senescence only after about 50 to 60 population doublings when grown *in vitro* [2], [3]. However, stem cells and cancer cells possess ability to eliminate such replicative barriers thereby acquiring potential to divide indefinitely *in* vitro [4]. The genetic alterations required to convert somatic cell into cancer cell have been characterized by many researchers. This conversion can take place by activation of oncogenic RAS, blockage of the p53/Rb pathways, activation of telomerase and many other aberrant pathways [5]. However, for cancer to metastasize and initiate angiogenesis there are many other alterations required [4], [6]. But because somatic cells undergo very few mutations and their lifespan is very less study suggests that alterations are multi-step process and takes place over many generations [7].

Stem cells and cancer cells both possess potential of rapid clonal proliferation, only the way they differs is the control on proliferations. Stem cells proliferate to give rise to complete organism or repair tissue/organ in highly controlled manner, where as cancer cell proliferation is uncontrolled and detrimental. Stem cells possess ability to get recruited, inhabited in various microenvironments of the human body; cancer cells also do possess this property evident by the process of metastasis [8]. These similarities in cancer and stem cells might help in finding out ways by which therapeutic interventions can be designed. This can be achieved by investigation of molecular pathways involved in self renewal, differentiation and tumorigenesis in cancer cells and stem cells.

The present article is based on literature survey in the said topic which includes major cell signalling pathways, their normal mechanism of action in stem cells and their mechanism of action in cancer cells. We believe analyzing normal and abnormal cell signalling pathways in stem cells and cancer cells will broaden our understanding of designing therapeutic interventions for various malignancies.

II. SIGNAL TRANSDUCTION PATHWAYS

Seven important signaling pathways reported to govern cell proliferation in stem cells as well as cancer cells [8], [9]. These seven common pathways are,

A. Wnt (Wingless-related integration site) Pathway

B. NOTCH Signaling Pathway

C. JAK/STAT (Janus kinase - Signal Transducer and Activator of Transcription) Pathway

D. MAPK (Mitogen-Activated Protein Kinases)/ERK (Extracellular signal-Regulated Kinases) Pathway

E. TGFβ (Transforming Growth Factor Beta) Pathway

F. NFkB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) Pathway

G. PI3K (Phosphatidylinositol 3-Kinase)/AKT Pathway

A. Wnt (Wingless-related integration site) Signaling Pathway:

Wingless-related integration site (Wnt) pathway is the signaling pathway consists of various proteins which serve as messengers to pass signals from outside of the cell to the inside through membrane piercing cell surface receptors. Wnt signaling pathway is highly evolutionarily conserved pathway among many species ranging from fruit flies to humans [10], [11]. Pathway is further branched into three different directions named the canonical Wnt pathway; the non canonical planar cell polarity pathway and the non canonical Wnt/calcium pathway [65].

The canonical Wnt pathway act by regulating gene transcription, the non canonical planar cell polarity pathway act to regulate cytoskeleton of the cell, while the non canonical Wnt/calcium pathway regulates calcium inside the cell. All these pathways are activated by Wnt-protein ligand binding to a Frizzled family cell surface receptor. This binding result into direct signal transmission from Frizzled receptor to cytoplasmic protein called Dishevelled (Dsh). Wnt signaling pathways work by either paracrine (nearby cell to cell communication) or autocrine (within the cell communication) effects.

❖ *Discovery:*

The title 'Wnt' is influenced by research done by number of scientists and is actually combination of two terms. One group of researchers in 1982 were working on oncogenic retroviruses by infecting mice with mouse mammary tumor virus to see which mouse genes possess potential to produce breast tumor upon mutation and claimed identification of new proto-oncogene called integration 1 (int1) [10], [12]. Later it was found that int1 is highly conserved across several species, including Drosophila and humans. In 1987, researchers determined that the int1 gene in Drosophila is actually already known and characterized known as Wingless (Wg). This fact at least proved that mammalian int1 was homologue of Wg [10]. This discovery of homology played an important role because previous work done by Nusslein-Vilhard and Wieschaus showed that Wg involved in embryonic development as a segment polarity gene [13]. For a long period of time it was considered that cancer is caused by aberrant embryonic development mechanisms; reason why there was abundance of research with respect to int1 and related signaling pathways. This further research lead to unleashing many other genes related to int1; but all those genes were not expressed by the activation mediated by proviral integration, it became clear that the nomenclature of the gene 'int' was not apparent. However, int/Wg family was then renamed as 'Wnt' and which literally means Wingless related integration site [10].

❖ *Mechanism of Action:*

The pathway starts when one of the Wnt proteins binds to the N-terminal extracellular cystein-rich domain of a Frizzled (Fz) family receptor [14] which are family of G-protein coupled receptors spanning the plasma membrane seven times [15]. There are almost 30 different extracellular Wnt proteins with affinity for Fz receptors [16]. There are also co-receptors involved in the process like LRP- 5/6 (lipoprotein receptor-related protein, Ryk (receptor tyrosine kinase), and ROR2 [17]. After this activation of receptor by Wnt, the signal is then transmitted to cytoplasmic phosphoprotein called Dishevelled (Dsh) via direct interaction. Dsh protein is highly conserved among all organisms with following three domains, an amino-terminal DIX domain, a central PDZ domain, and carboxy-terminal DEP domain. The different combination of these three domains guides different branches of pathways leading to activation of different genes [18].

Out of three branches of Wnt signaling pathways, the canonical pathway is found to be involved in tumorigenesis as well as in maintenance of pluripotency in stem cells. The canonical Wnt pathway is involved in number of tumors like colon and breast cancers. It is also the same pathway which is responsible for maintaining pluripotency in all types of stem cells.

❖ *The Canonical Wnt Pathway/β-catenin Pathway:*

There are two scenarios in this, the pathway in the absence of Wnt ligand at the Fz receptor and the pathway when Wnt ligand binds to the Fz receptor. In the absence of Wnt protein ligand at Fz receptor **(absence of Wnt signaling),** β-catenin does not gets accumulated in the cytoplasm, eventually does not get translocated into nucleus which otherwise would have led to genes to express their protein products (See Fig.1) [66]. The reason behind non-accumulation of β-catenin in cytoplasm is being degradation of β-catenin by destruction complex triggered by absence Wnt signaling. The destruction complex composed of number of proteins like APC (adenomatosis polyposis coli), Axin, GSK3 (glycogen synthase kinase 3), PP2A (protein phosphatase 2A) and CK1α (casein kinase-1α). This destruction complex induces β-catenin degradation by phosphorylation & ubiquitination and then sends it to proteosome for further digestion [14, 19].

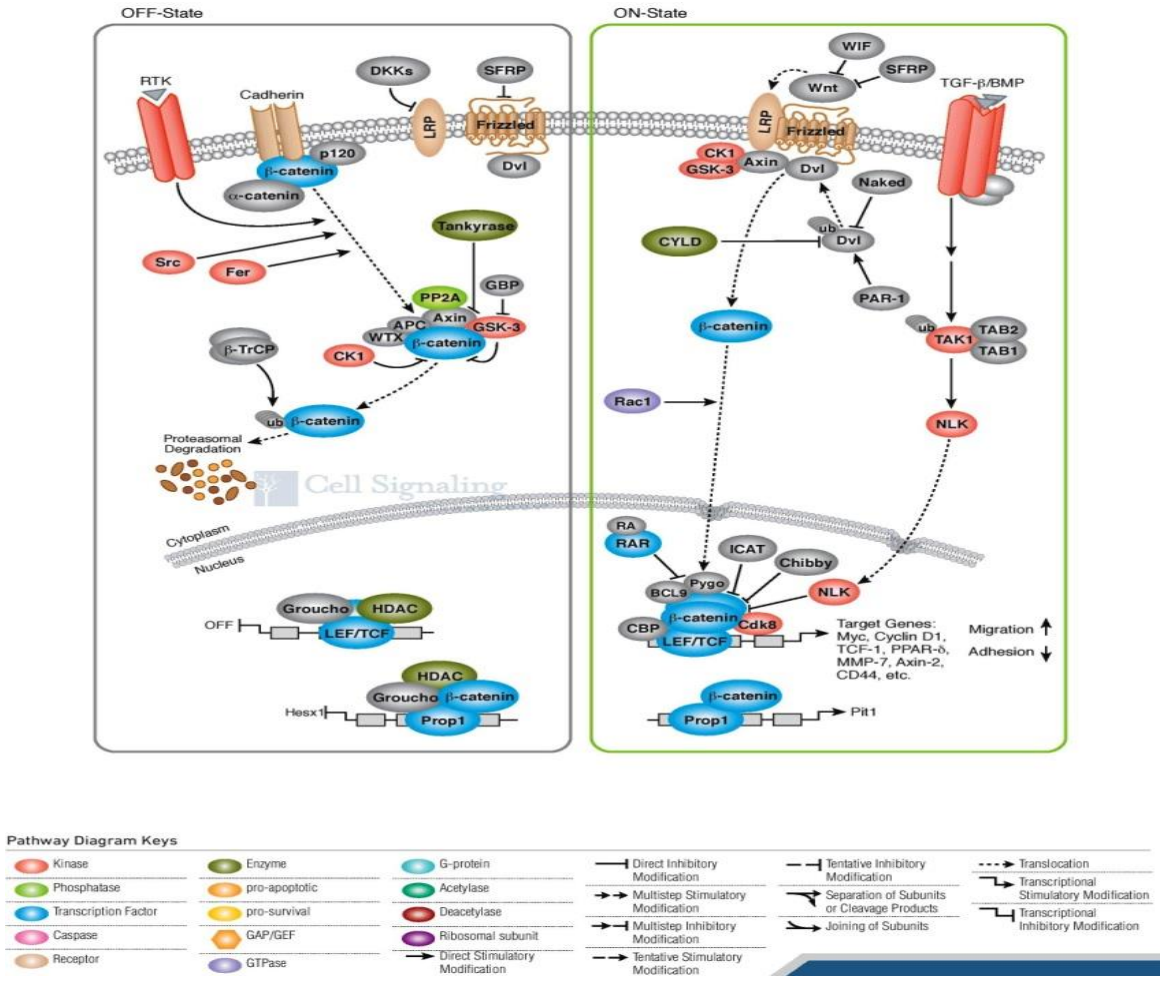

Figure 1) Canonical Wnt Signaling Pathway (ON & OFF state)

Wnt signalling pathway in OFF (absence of Wnt ligand) state and ON (presence of Wnt ligand) state. Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

In the presence of Wnt protein ligand at Fz receptor **(presence of Wnt signaling)**, the destruction complex gets deactivated by inhibiting GSK3 protein. Inactivation of destruction complex results in accumulation of β-catenin in the cytoplasm which eventually translocated into nucleus where it acts as co-activator for transcription factors that belongs to TCF (T cell factor)/LEF(Lymphocyte enhancer binding factor) family. This transcription factor activated by β-catenin results into initiation expression of gene such as c-myc and cyclin D1 [20].

In mammals Wnt signaling found in stem cells (both embryonic and adult stem cells) plays important role in maintaining self-renewal [21]. Cytoplasm and nucleus of adult stem cells of hair follicle and skin is enriched in β-catenin, here β-catenin functions in self-renewal and lineage determination. One study showed that if β-catenin gene deleted in mice resulted into impairment of hair follicle morphogenesis and loss of follicle stem cell microenvironment [22]. Wnt signaling is pre-requisite for maintaining stem cell Pluripotency and mutation in the pathways linked to variety of epithelial carcinomas [8].

❖ ***Wnt Signaling in Cancer Cells:***

Fine balance between dying and newly forming cells in the tissues and organs of human body is maintained by process called homeostasis. Replacement of old cells with new ones is a continuous process and the frequency varies according to tissue under consideration. Epithelial lining intestine is replaced on weekly basis; interfollicular epidermis is replaced every month, whereas lung epithelia are replenished every six months [22].

The colon is two-layered sheet of cells; the bottom layer consists of proliferating stem and precursor cells, while the upper layer consists of differentiated non-dividing epithelial cells. The bottom layer of highly proliferative stem cells maintains epithelial homeostasis by continuously replenishing them with fresh cells. The status of Wnt signaling in bottom layer

stem cells is 'ON', however, as differentiation progresses and these cells migrates to the top layer the signaling gets "OFF' in completely differentiated epithelial cells. It is observed that in about 90% colon cancers, β-catenin is stabilized, accumulated and initiated TCF/LEF transcriptional program through mutation in one of the destruction complex protein 'APC' (APC in normal pathway targets β-catenin resulting into its degradation) [23]. Thus, absence of or abnormal APC leaves destruction complex inactive resulting in β-catenin mediated continuous replication of cells. This also poses risk of further mutations which makes these cells malignant [24]. A mutation in APC or β-catenin resulting in up-regulation of Wnt pathways is also evident in gastric polyps (about 64% cases) and intestinal adenocarcinomas (about 48% cases) [23]. Chronic and acute myeloid leukaemia is found to be caused due to faulty Wnt signalling [24], [25].

B. NOTCH Signaling Pathway:

This highly conserved pathway is present in all metazoans and in most of the multicellular organisms [26]. Mammals possess four different types of notch receptors namely NOTCH1, NOTCH2, NOTCH3 and NOTCH4. NOTCH is the transmembrane receptor protein which passes through membrane at single point only. NOTCH receptor is with two different portions (hetero-oligomer), extracellular and intracellular portion. These two portions attaches with each-other by noncovalent interaction which is calcium dependent and connected by single-transmembranepass [27]. NOTCH signaling is important and induces neurogenesis and it is inhibited by protein 'Numb' to promote neural differentiation.

❖ *Mechanism of Action:*

The NOTCH pathway consists of NOTCH receptor, NOTCH ligands and proteins in the cell to transmit signal to the nucleus. In Drosophila and *C. elegnas*, the NOTCH/Lin-12/Glp-1 receptor family found to be responsible for specification of cell fates during development [28], [29]. NOTCH receptor is transmembrane protein with extracellular and intracellular parts. Ligand proteins bind to the extracellular region of NOTCH promotes proteolytic cleavage and subsequent release of intracellular (NICD) part (See Fig. 2) [67]. The cleavage is mediated by ADAM-metalloproteases and γ-secretase. The extracellular part remains in place while the intracellular part (NICD) finds its way into the nucleus and alters gene expression [30] by forming transcriptional activation complex with the DNA-binding factors CSL (CBF1/CBF1/Suppressor of Hairless/Lag1) and the Mastermind-like protein family (MAML).

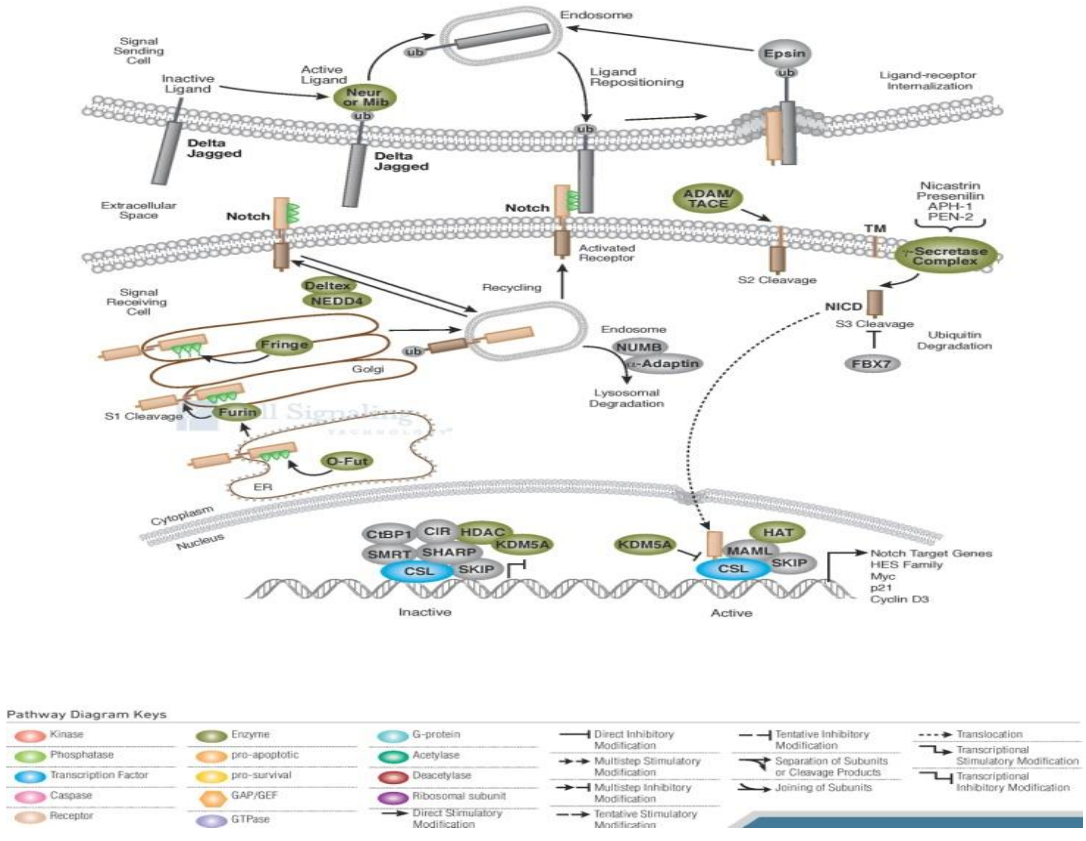

Figure 2) NOTCH Signaling Pathway

Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

NOTCH signaling operates by the mechanism of direct cell-to-cell contact since most of the ligands which are having affinity for NOTCH receptor are transmembrane proteins expressed on other cells. This helps group of cells in organizing and forming large structures, NOTCH signaling also let adjacent cells know which genes are active in one cell and need to switch off in another and do so by NOTCH signaling. For example, during tissue development this pathway play crucial role in mediating signals between adjacent cells. NOTCH signaling mediates the inhibition of spread of cellular differentiation within the tissue called lateral inhibition or it promotes adjacent cells to acquire differentiation called lateral induction [31]. NOTCH signaling pathway plays significant role in cell-cell communications which in turn act to regulate multiple cell differentiation processes in embryonic and adult stage.

NOTCH signaling is not a pre-requisite to maintain pluripotency in stem cells, because it was found that NOTCH signaling is inactive in undifferentiated human embryonic stem cells [32]. However, NOTCH signaling is a pre-requisite of differentiation and cell fate decision and aberrant regulation of this pathway has been found in many human cancers.

❖ ***NOTCH Signaling in Cancer Cells:***

It has been found that the aberrant NOTCH signaling is associated with breast tumors, medullablastoma, ovarian cancers, and melanoma progression. The more aggressive phenotype in human breast tumors is correlated with amplification of NOTCH receptors and presence of Jagged 1 type of ligands. However, expression of protein 'Numb' (NOTCH antagonist) can revert this phenotype [33]. It is implicated that NOTCH might increase cell proliferation by activation of its downstream target c-myc. c-myc is transcription factor which is mis-regulated in many cancers.

In T-ALL (T-cell acute lymphoblastic leukemia/lymphoma), uncontrolled proliferation of immature T cells leads to formation of blood cancer [34]. In approximately 55 to 60% T-ALL cases, mutation in NOTCH signaling pathway has been seen. However, it is not clear whether tumorigenesis is a result of these mutations or they are secondary events during aggressive proliferation. In mouse model it is proved that NOTCH is crucial in the early developmental stage of T cell and also play role in further differentiation of T cells to intrathymic T cells. These results indicate that if NOTCH signaling remains active beyond normal development may result into neoplasia character [35].

C. JAK/STAT (Janus kinase - Signal Transducer and Activator of Transcription)Pathway:

This pathway is responsible for transmitting chemical signals from outside of the cell, through the cell membrane, into the cell, especially into gene expression promoters on the DNA. This results into initiation of DNA transcription and further gene expression. JAK/STAT pathway is a major signaling alternative to second messenger system. Second messenger system composed of secondary messengers which are the components of signal transduction cascade. This pathway predominantly functions in white blood cells, reason why it is involved in regulation of the immune system. It is equally important in mediating cell fate, like apoptosis, differentiation and proliferation in response to growth factors and cytokines.

The JAK/STAT signaling system is with three major components, receptor, Janus kinase and signal transducer and activator of transcription (STAT) [36].

The pathway initiates when the cell surface receptor gets activated by the stimuli of interferon, growth factors, interleukin or other chemical messengers. This results into activation of kinase of JAK which subsequently phosporylates itself (autophosphorylation). Phosphate groups here acts as 'on' 'off' switches on protein. STAT protein binds to phosphorylated receptor resulting in STAT phosphorylation by JAK. The STAT then dimerizes by binding to another phosphorylated STAT. This phosphorylated STAT dimer migrates to nucleus where it binds to DNA and promotes gene transcription.

STAT action is very specific, in mammals there are seven STAT genes. Each one is very specific in binding to the specific DNA sequence only. STAT binds to DNA promoter which controls the expression of other DNA stretches also. This leaves effect on basic cell functions, like growth, differentiation, proliferation and death [36].

❖ ***Mechanism of Action:***

JAK (Janus kinase) possess tyrosine kinase activity and binds to some cell surface cytokine receptors. Binding of cytokine ligand to the receptor activates JAK. JAK phosphorylates tyrosine residues present on the receptor. The process also creates sites for interaction with proteins containing SH2 domains which possess affinity for phosphortyrosine residue. STATs possess SH2 domains which bind to this phosphor-tyrosine residue and get recruited to the receptors. After this binding STATs themselves gets phosphorylated by JAK. The same process at other JAK results into dimerization of

STAT forming homo- or heterodimers. STAT dimers so formed become active and translocates to the nucleus. This translocation accumulates STAT dimers in the nucleus and eventually activates transcription of their target genes [37] (Refer Fig. 3) [68].

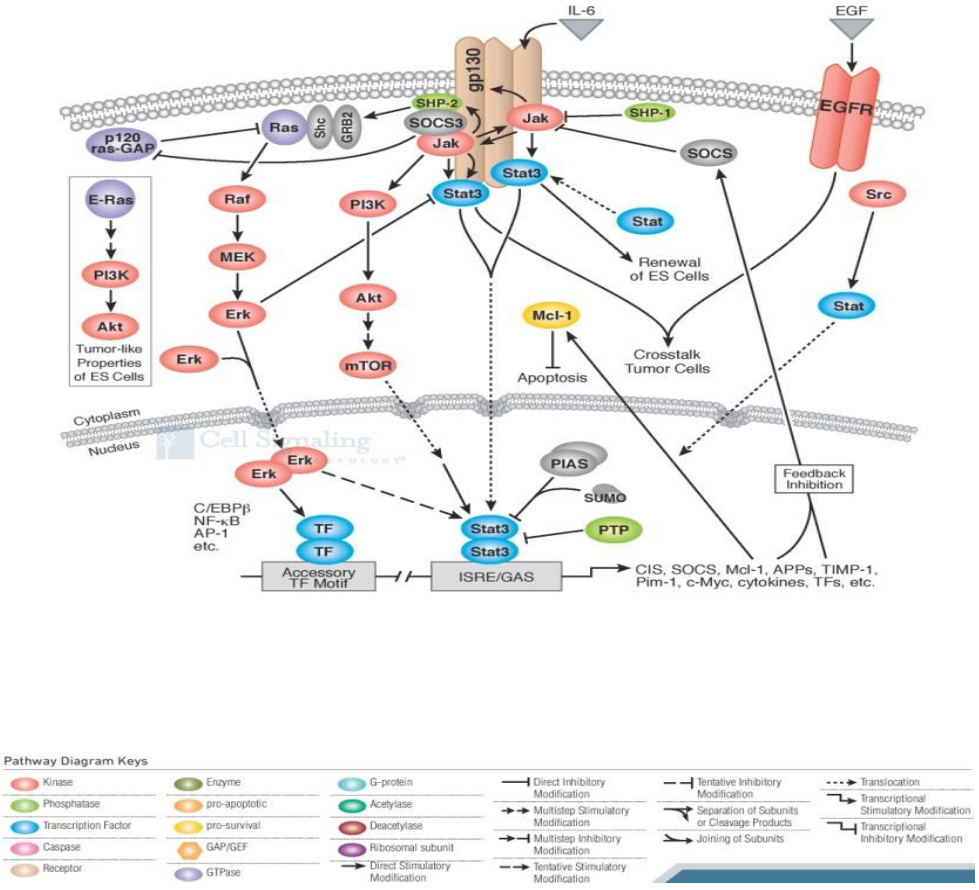

Figure 3) JAK/STAT Pathway

Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

The JAK/STAT pathway is evolutionarily conserved from slime molds to mammals except fungi and plants. Aberrant JAK/STAT behaviour is found to contribute to immune deficiency syndromes and cancers [36].

❖ ***JAK/STAT Pathway in Cancer:***

The pathway is implicated in apoptosis, growth factor signaling and cellular immune response. Mis-regulated or aberrant JAK/STAT pathway may results into tumorigenesis directly or indirectly [38]. Fusion, amplification or mutations in any protein component of pathway can cause hypersensitivity to mitogenic signals and elevate rate of proliferation. These pathway components may include EGF-R (Epidermal growth factor-receptor) in brain, breast and stomach tumors, or HER2/neu- in mammary and stomach carcinomas [39], [40]. The constant activation of STAT3 has been implicated in more than 95% head and neck cancers, and in more than 50% of lung and breast cancers [41]. Improper behaviour of this pathway alters the way cells respond to cytokines. For example, aberrant JAK/STAT indirectly results into tumor formation by compromising tumor immune surveillance.

D. MAPK/ERK (Mitogen-Activated Protein Kinases/Extracellular signal-Regulated Kinases) Pathway:

MAPK or ERK pathway is crucial in transmitting cytokine signal from extracellular region into the cell through cell surface receptor tyrosine kinase and plays important role in cell adhesion, proliferation, migration and survival [42]. The membrane-bound GTPase, RAS, is central molecule of this pathway. RAS, depending on signal is either inactive GDP-bound or in active state by binding to GTP. But RAS is not directly activated by signal; it is stimulated by SOS (Son of Sevenless). SOS is found in cytoplasm and is a guanine exchange factor. Once signal is passed by receptor, SOS moves to the cell membrane where it catalyzes the nucleotide exchange of RAS. This converts inactive RAS to GTP bound active RAS which in turn activates the serine/threonine kinase RAF. RAF then activates Mitogen-activated protein kinase

(MAPK) or also called extracellular signal regulated kinase (ERK). ERK moves into the nucleus where it activates the Jun/Fos transcription factors.

Mutation is in any of the constituent protein of the pathway leads to either pathway 'on' or 'off' state. This blockade is one of the pre-requisite in developing malignant phenotype. In fact, constituent proteins of MAPK/ERK pathway were discovered when researchers found their role in cancer development. Many drugs are under investigation which targets this 'on'/'off' switch for cancer therapy [43].

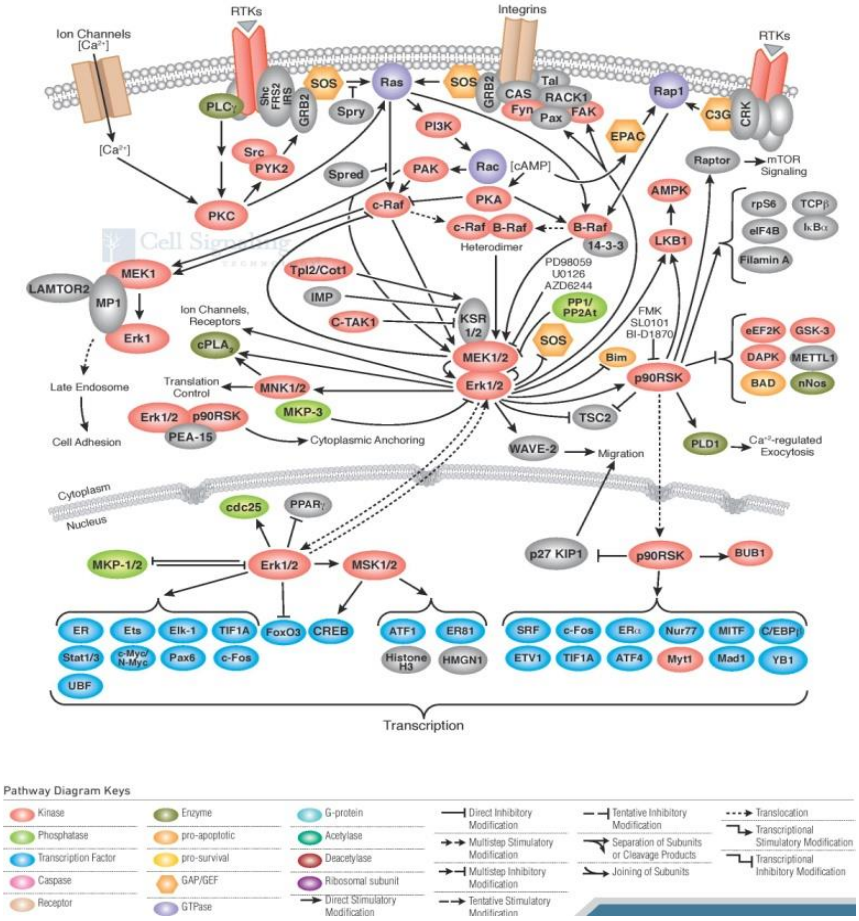

Figure 4) MAPK/ERK Pathway

Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

❖ ***MAPK/ERK Pathway in Cancer:***

Aberrant MAPK/ERK pathway is implicated in many cancers since this pathway plays crucial role in cell proliferation. The hyper-proliferative state is acquired by cells in which RAS and RAF proteins are mutated leading to pathway switch kept 'on'. RAS, which is the constituent protein of SOS-Ras-Raf-MAPK signaling cascade, have been found mutated in almost 45% colon cancer cases and about 90% of pancreatic cancer cases [42]. Many melanoma cases have been implicated to mutation in RAF [44]. Since these pathway constituents are involved in many cancers, they are ultimate targets for therapeutic intervention.

E. TGFβ (Transforming Growth Factor Beta) Pathway:

This pathway is found in many cellular processes governing cell growth, differentiation, apoptosis and plays critical role in maintaining cellular homeostasis of tissue/organ in adults as well as in embryos. TGFβ pathway is evolutionarily conserved and is quite complicated pathway in metazoans. There are 42 TGFβ superfamily ligands which bind to type-2 receptor. This induces phosphorylation and recruitment of type-1 receptor for phosphorylation of receptor-regulated SMADs (R-SMADs) which in turn bind the coSMAD SMAD4. SMADs are transcription factors. The complexes of R-SMAD/coSMAD accumulate in the nucleus where they act as a transcription factors and regulates target gene expression.

The pathway is further divided into three main branches as; the SMAD1/5/8, the SMAD2/3 and the TAB/TAK branch.

❖ ***Mechanism of Action:***

This pathway was first discovered as anti-proliferative signal to keep check on cell proliferation thereby achieving homeostasis. Cell growth inhibitory signals appears as soluble factors in the extracellular region or gets expressed on the surface of nearby cells and causes cell to undergo reversible growth arrest, the process called quiescence. The TGFβ pathway is important in doing this function.

The pathway gets activated when TGFβ ligands attaches to extracellular domains of type-1 and type-2 TGFβ receptors. Serine phosphorylation transmits signal to cytoplasmic transcription factors called SMADs. Type-1 receptors ALK1, ALK2, ALK 3 and ALK6 possess affinity for Bone Morphogenic Protein (BMP) and Growth Differentiating Factor (GDF) ligands which leads to phosphorylation and activation of SMAD1 and 5. However, phosphorylation and activation of SMAD2 and SMAD3 is achieved by binding of Activin and Nodal ligands to ALK4, ALK5 and ALK7 receptors (See Fig. 5) [69]. Phosphorylated SMADs forms complex with SMAD4 and moves to nucleus where they regulate transcription of their target genes.

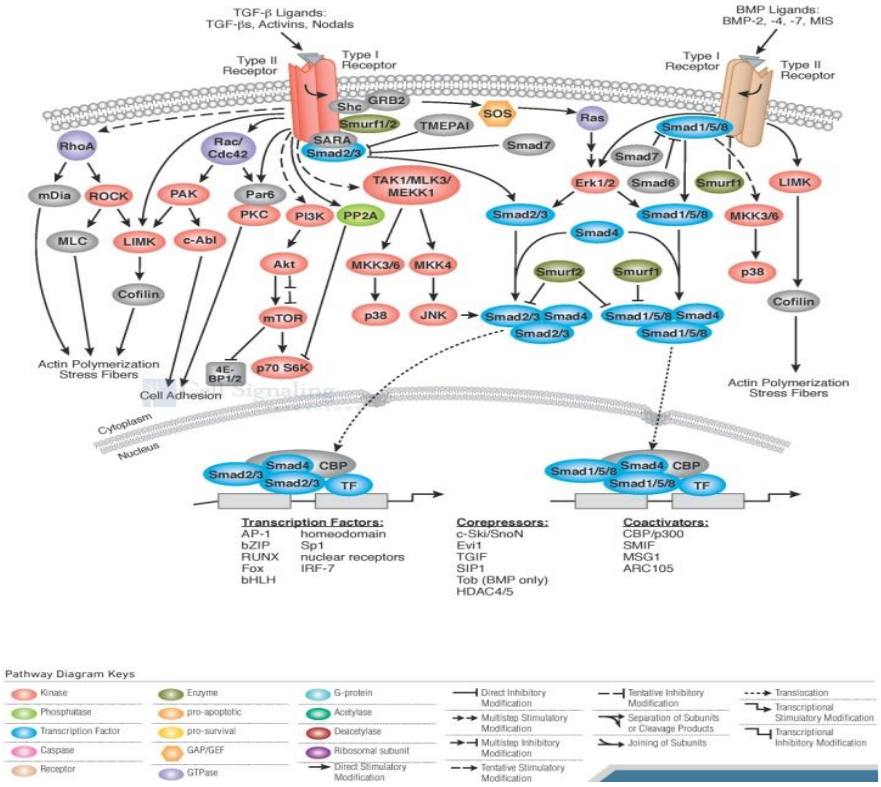

Figure 5) TGFβ Signaling Pathway

Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

❖ ***TGFβ Pathway in Cancer:***

It has been observed that one of the component proteins of the pathway, BMP2, is over-expressed in almost 98% of lung carcinomas [45], [46]. Tumor growth is reduced by using BMP-antagonist called Noggin [45]. It is also clear that BMP2 does not promote growth of subcutaneous tumors and it has been demonstrated that it inhibits growth of some cell line *in vitro* [46]. This fact is suggestive of dependency of BMP2 on some other factors for growth promoting properties.

Other than this, mutation or down regulation of TGFβ receptor or inactivation of SMAD4 is also implicated in number of cancers, especially; inactivation of SMAD4 is evident in about 53% of human pancreatic ductal adenocarcinomas [47], [48]. TGFβ signaling pathway act as 'on' or 'off' switch for regulation for cell proliferation and is an important in metastasis [49].

F. NFkB (Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells) Pathway:

NFkB (Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells) is protein complex involved in transcriptional control of particular genes and is responsible for cytokine production and cell survival. It is found in all animal cell types

and it make cells to respond to stimuli like stress, free radicals, cytokines, oxidized LDL, UV irradiation and bacterial or viral antigens [50], [51], [52], [53], [54]. Notably, kappa light chains are crucial components of immunoglobulins and hence this pathway plays important role in regulating immune response to infections by innate immunity and inflammation [9], [50].

❖ **Mechanism of Action:**

Transcriptional regulation of many target genes is achieved by NFkB transcriptional factors family which consists of p52/p100, p50/p105, RelA, RelB and c-Rel. Proteolysis of P105 and P100 gives rise to p50 and p52 respectively. However, RelA, RelB and C-Rel possess C-terminal trans-activation domain and are not processed. RelA, RelB and C-Rel, p50 and p52 forms homo- or heterodimers and moves to nucleus where they bind to DNA regulatory kB sites. Absence of signaling leads to accumulation of NFkB dimers in the cytoplasm and gets inactivated by interacting with I-kB inhibitory proteins. In response to extracellular stimuli like TNF- α (Tumor Necrosis Factor-α), interleukin-1, bacterial or viral infections, growth factors and oxidative stress, I-kB undergoes rapid phosphorylation on serine 32 and 36 by the IKK (I-kB kinase). This phosphorylated I-kB is then directed to ubiquitination by ubiquitin ligase complex and targeted for degradation by the 26S proteosome. The released dimers then translocate to the nucleus and activate transcription of target genes (See Fig. 6) [70].

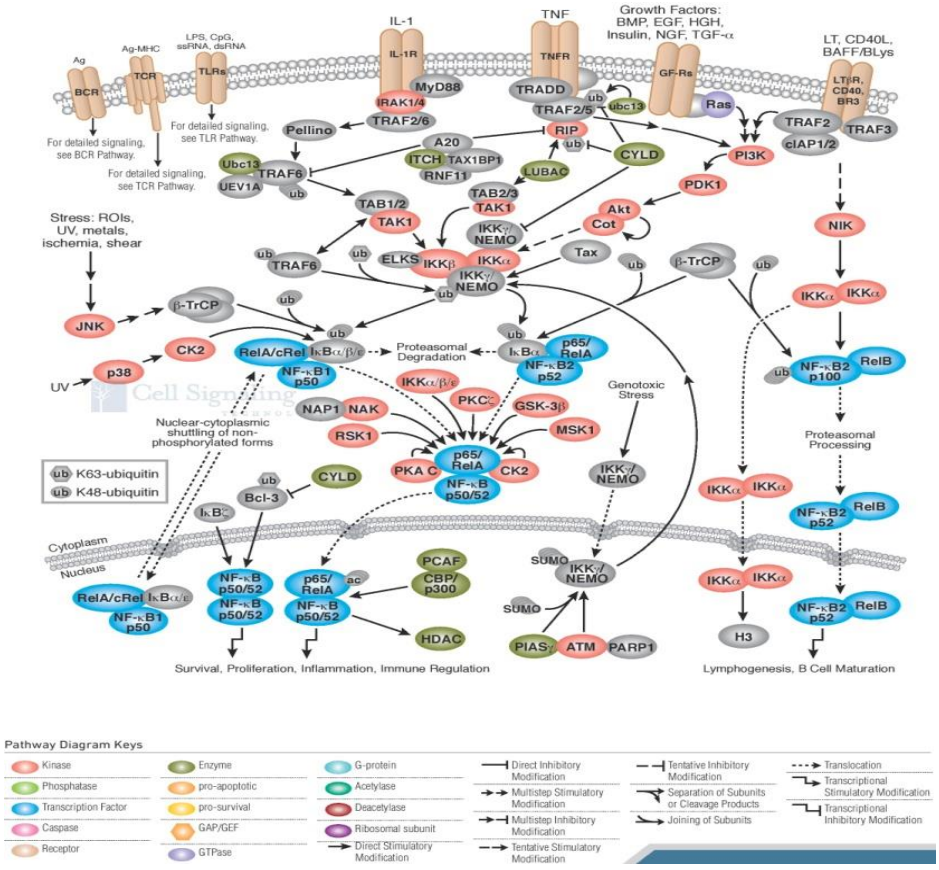

Figure 6) NFkB (Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B cells) Pathway

Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

❖ ***NFkB Pathway in Cancer:***

Aberrant regulation of this pathway not only implicated in the process of synaptic plasticity and memory but in inflammatory and autoimmune diseases, improper immune system development, viral/bacterial infections and many cancers [55], [56], [57], [58], [59]. For example, in B cell malignancy, human REL gene which codes for one out of the five transcription factors is found to be over-expressing in about 10 to 20% of non-Hodgkin's B cell lymphomas, about 40% of KN T cell lymphomas and about 50% of Hodgkin's lymphomas. This over-expression of protein by this gene suggested outcompeting inhibitory action imposed by I-kB in the cytoplasm which results into constitutive transcription

of NFkB target genes and increase in B cell proliferation and survival. Immunohistochemistry of lymphoma samples derived from patients with REL over-expression confirmed nuclear REL in some cases [60].

G. PI3K (Phosphatidylinositol 3-Kinase)/AKT Pathway:

PI3K are group of enzymes involved in functions such as cell growth, proliferation, differentiation, motility, survival and intracellular trafficking and hence are also implicated in cancers. These intracellular signal transducer enzymes with potential of phosphorylating the 3 position hydroxyl group of the inositol ring of phosphatidylinositol. They are also called phosphoinositide 3-kinases. Interaction of PI3Ks with Insulin receptor substrate (IRS) leads to regulation of glucose uptake through events of phosphorylation. PI3K family is further branched in to three classes namely, Class-I, Class-II and Class-III. The classification primarily based on protein primary structure, regulation and *in vitro* lipid substrate specificity [61].

❖ *Mechanism of Action:*

The pathway is responsible to respond to various intra- and extracellular signals through RTK (transmembrane tyrosine kinase-linked receptors) and with the help of various intracellular factor which results into regulation of cytoskeletal rearrangements, cellular proliferation and death [62]. Heterodimeric Class-I PI3Ks composed of two subunits; adaptor / regulatory p85 subunit and a p110 catalytic kinase subunit. Various activating proteins like RHO, mutated RAS, SRC, protein kinase C interacts with p85 subunit of the Class-I PI3K and results into activation of p110 catalytic kinase subunit. Once activates, PI3K phosphorylates PIP2 (phosphatidylinositol-4, 5-biphosphate) at a 3-position, converting it to PIP3 (phosphatidylinositol-3,4,5-biphosphate). PI3K/AKT signaling is counteracted by the Phosphatase and PTEN (TENsin homologue) (and SHIP1, SHIP2), which dephosphorylates PIP3–PIP2. The AKT protein is one of the critical downstream mediators of PIP3 with high affinity for PIP3 through its Pleckstrin Homology (PH) domain. Activated AKT is then moved to the cell membrane interacting with PDK1 (Phophoinositide Kinase 1) and gets phosphorylated. Activated AKT then regulates variety of downstream targets and implicated in number of human diseases. In mouse embryonic stem cells, this pathway is critical in maintaining pluripotency [63].

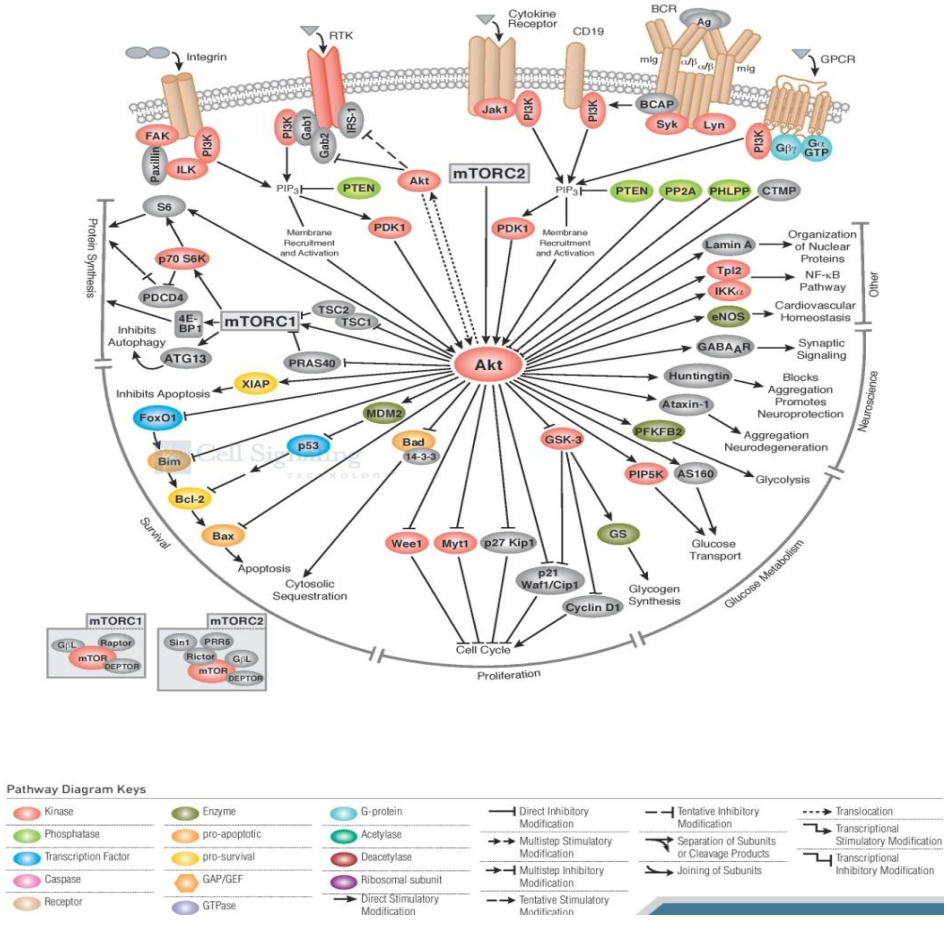

Figure 7) PI3K (Phosphatidylinositol 3-Kinase)/AKT Pathway

Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

❖ ***PI3K (Phosphatidylinositol 3-Kinase)/AKT Pathway in Cancer:***

Since PI3K/AKT pathway continuously communicates to other pathways like apoptosis pathways, NFkB pathway and Wnt pathway it is suggestive of importance of this pathway in forming malignancy. There are number of downstream proteins which get activated once they receive PI3K signal and leads to cellular metabolism, proliferation and survival. It has been reported that directly or indirectly the activated AKT pathway inhibits apoptosis mediators such as BAD and pro-caspase 9 [64]. AKT pathway found to be involved in the direct activation of negative regulator MDM2 of p53. p53 is an important component which checks DNA for damage, initiates cell cycle arrest and DNA repair. AKT pathway also inhibits a negative regulator (Glycogen Synthase Kinase-3) of Wnt pathway. Poorly regulated PI3K/AKT pathway has been implicated in many cancers. For example, lung cancer, melanomas and glioblastomas are caused due to mutation in pathway inhibitor and tumor suppressor PTEN, and in thyroid, ovarian and many other cancers pathway have been found to be over-activated [64]. The constitutive activation of this pathway in many cancers induces cell survival and proliferation. PI3K pathway also stimulates other pathways like Wnt and NFkB to participate in cell proliferation.

III. CONCLUSION

All above seven pathways are found to be involved in both; maintaining 'stemness' (in stem cells) and in making cell malignant (in cancer cells). When components of any of these pathways over-express or deregulate results into aberrant pathway which in turn exerts its effect on cell proliferation and loss of cellular homeostasis. These pathway components have been targeted for therapeutic interventions by many drugs in combination with other therapies. These drugs specifically target faulty pathway component in an attempt to revert malignant cell type into normal. Many monoclonal antibodies also utilized based on the same concept. However, it is interesting to investigate whether normal pathways in stem cells can revert cancer cells into normal cells by any of the mechanisms like direct cell-cell communication or paracrine effect. This facet of the stem cell potential remains un-evolved and is challenge for research community.

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