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## Dysregulation of Signaling Pathways Plays a Role in the Development and Pathogenesis of Hepatocellular Carcinoma

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### Authors' contributions

This work was carried out in collaboration between all authors. Authors RHA and AZE designed the study, wrote the protocol, managed the literature searches, edited the manuscript and supervised the work. Author DADA collected data and wrote the first draft of the manuscript. Authors HAEA and MAMNA revised the manuscript. All authors read and approved the final manuscript.

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### ABSTRACT

**Background:** Hepatocellular carcinoma (HCC) is the fifth most common solid malignancy worldwide and causes more than 600,000 deaths annually. Many risk factors predispose to HCC, these risk factors may present individually or collectively depending on the environmental situations. The risk factors for HCC include hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholism, aflatoxin (AF), schistosomiasis and some hereditary diseases as haemochromatosis and haemophilia. Certain key regulatory signaling pathways integrated in the pathogenesis of HCC such as insulin-like growth factor (IGF) signaling pathway, transforming growth factor- $\beta$  (TGF- $\beta$ )

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signaling pathway, Toll-like receptor (TLR) signaling pathway and Notch signaling pathway. Dysregulation of different signaling pathways at any level including the receptors and downstream signaling pathway components represent a central protumorigenic principle in human hepatocarcinogenesis and this dysregulation can be targeted for new therapeutic modalities for HCC.

**Aim of the Work:** The aim of the present work was to study the recently discussed signaling pathways integrated in development of hepatocellular carcinoma in the high risk groups, and how genetic alterations in these signaling pathways is a potentiating factor for HCC from the year 2000 until now. New diagnostic and therapeutic modalities for that disease could be provided by targeting these dysregulated signaling pathways.

**Conclusion:** Finally, it could be concluded that: 1) There are multiple mechanisms underlying the hepatocarcinogenesis and by these mechanisms, early diagnosis for HCC could be possible. 2) Understanding the signaling pathways dysregulated in high risk groups for HCC may provide new diagnostic and therapeutic modalities.

*Keywords: Hepatocellular carcinoma; dysregulation; signaling pathways.*

## 1. SIGNALING PATHWAYS INTEGRATED IN THE PATHOGENESIS OF HCC

Hepatocarcinogenesis is associated with a large accumulation of chromosomal, genetic and epigenetic alterations. Some of these alterations occur in different stages of hepatocarcinogenesis and the cause of these alterations could be dysregulation of important molecular cellular signaling pathways. Therefore, a malignant hepatocyte may be produced by alteration in gene(s) caused by dysregulation of different regulatory pathways [1].

Normal embryogenesis, organ development as well as tissue proliferation, regeneration and repair require balance of various molecular signaling pathways. Dysregulation of these signaling pathways and their components is the central principle in human tumorigenesis [2].

### 1.1 The Signaling Pathways Integrated in HCC Pathogenesis

There are multiple regulatory pathways with alterations that may have a role in HCC; these signaling pathways can be classified into:

- 1- Liver stem cell signaling pathways for example: TGF- $\beta$ , Wnt/ $\beta$ -catenin, IGF, TLR, Notch, Hepatocyte Growth Factor (HGF) and TGF- $\alpha$ /EGFR signaling pathways [2].
- 2- Signaling pathways that are associated with other types of cancers such as: nuclear factor-kappa B (NF- $\kappa$ B), janus kinase / signal transducer and activator of

transcription (JAK/STAT) and mitogen activated protein kinase (MAPK) signaling pathways. All these are known as key contributory pathways to the cell transformation, proliferation, apoptosis and invasive behaviors in human HCC [3]

#### 1.1.1 Liver stem cell signaling pathways

##### 1.1.1.1 Transforming growth factor-beta signaling pathway

TGF- $\beta$  is involved in cell differentiation, proliferation, migration and apoptosis. In the liver, TGF- $\beta$  is a potent growth inhibitor of normal hepatocytes and induces apoptosis and cellular senescence in these cells [4].

The basic signaling cascade of TGF- $\beta$  involves type I and type II transmembrane serine/threonine kinase receptors they are called TGF- $\beta$  receptor (TBR I and TBR II). Activation of these receptors is caused by TGF- $\beta$  factor binding to TBR leading to phosphorylation of the intracellular part of the receptor. The phosphorylated receptor recruits and activates intracellular signaling proteins by phosphorylation; these signaling proteins are called mothers against decapentaplegic protein (Smad) [5].

In vertebrates possess at least nine Smad proteins categorized into three functional classes: (1) Receptor activated Smads (R-Smads) as Smad1, Smad2, Smad3, Smad5 and Smad8. (2) Co-mediator Smads: Smad4 and Smad10 and (3) Inhibitory Smads: Smad6 and Smad7 [6].

Activation of TGF- $\beta$  signaling pathway results in association of R-Smads as Smad 2 and Smad 3 (after their phosphorylation) with Smad 4; this Smad complex then translocates to the nucleus where it interacts with various transcriptional factors such as cAMP response element binding protein (CREB) as shown in Fig. 1. These sequence of events leads to the enhancement of certain genes transcription such as p15 gene, p21 gene and E-cadherin gene [7].

There is an adaptor proteins named embryonic liver fodrin (ELF) these proteins play a critical role in modulating TGF- $\beta$  signaling. ELF is a dynamic scaffolding protein that is required for Smad3 and Smad4 localization (from the cytoplasm to the nucleus). Disruption of ELF results in mislocalization of Smad3 and Smad4 leading to loss of the TGF- $\beta$  dependent transcriptional response [9].

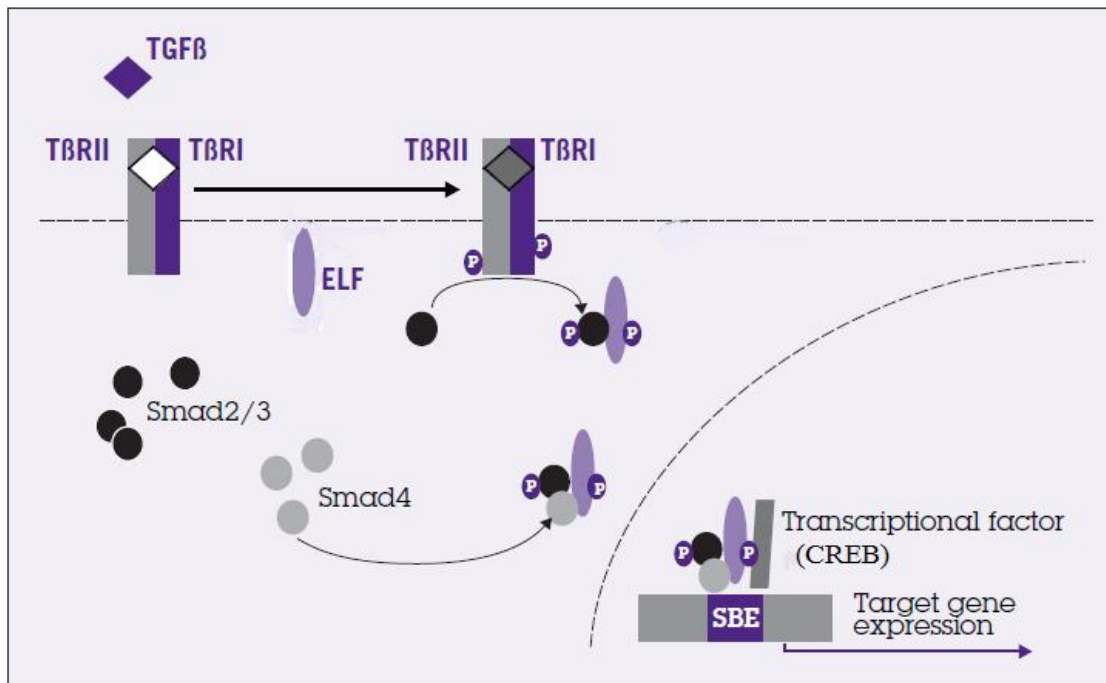
#### 1.1.1.2 Wnt / $\beta$ -catenin signaling pathway

Wnt signaling pathway plays a pivotal role in cell proliferation, cell/cell interactions, motility and tissue development [10]. Initiation of Wnt signaling involves the binding of Wnt proteins to a frizzled receptor (Fzd) and low density

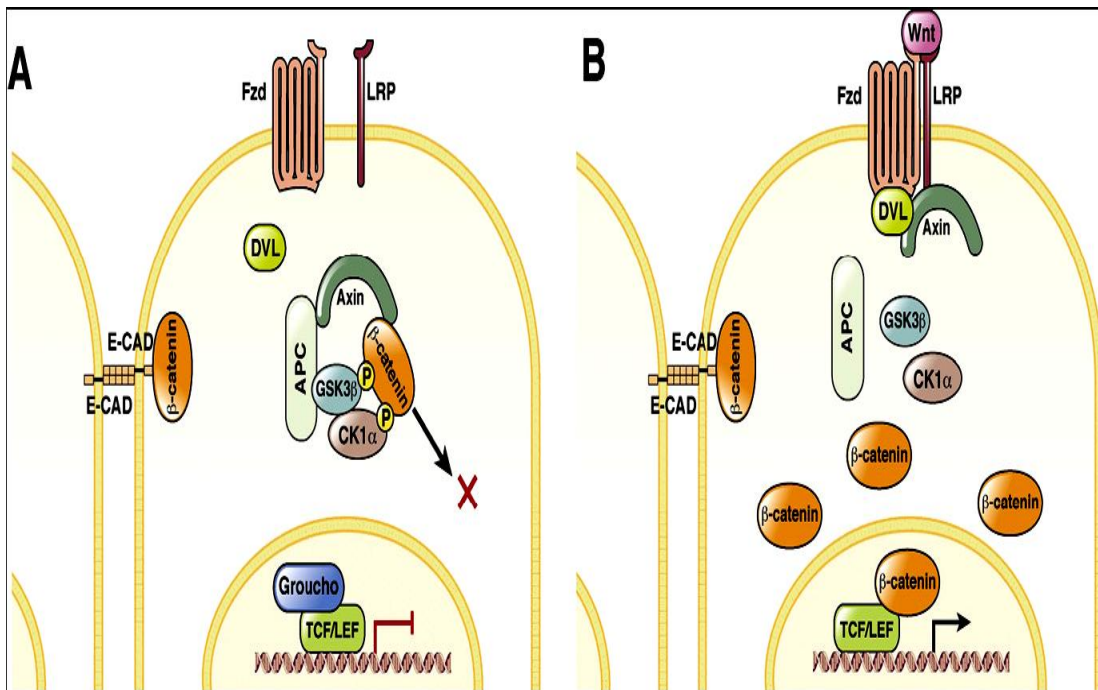
lipoprotein receptor related protein (LRP). The key intracellular component of this signaling pathway is cytoplasmic  $\beta$ -catenin protein [11].

In the absence of activated Wnt/ $\beta$ -catenin signaling, cytosolic  $\beta$ -catenin is rapidly phosphorylated by a complex of proteins collectively termed the “destruction complex” composed of the following proteins: Axin, adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3) and casein kinase 1 (CK1). The destruction complex phosphorylates the N-terminus of  $\beta$ -catenin, targeting the protein for proteasomal degradation and thereby maintaining low baseline cytosolic level of  $\beta$ -catenin [12].

The binding of Wnt ligand to Fzd receptor causes recruitment of the cytoplasmic dishevelled protein (DVL). The DVL protein causes disruption of the destruction complex leading to accumulation of cytoplasmic  $\beta$ -catenin.  $\beta$ -catenin, translocates to the nucleus where it serves as a coactivator to a number of genes by interacting with a family of transcription regulatory proteins called T-Cell Factor/ Lymphocyte Enhancer Factor (TCF/LEF) as shown in Fig. 2 [13].



**Fig. 1. Transforming growth factor-beta signaling pathway**  
(SBE: Smad binding element) (Quoted from Kitisin et al. [8])



**Fig. 2. Active and inactive Wnt signaling pathway. (A) In the absence of a Wnt ligand,  $\beta$ -catenin is bound to E-CAD at adherens junctions or is phosphorylated by the destruction complex. (B) Wnt ligand initiates signaling through FZD receptor and LRP coreceptor, activating and recruiting DVL and Axin to the membrane, thereby disrupting the destruction complex**  
(Quoted from White et al. [13])

Binding of  $\beta$ -catenin to TCF/LEF leads to stimulation of transcription of Wnt target gene to increase cell Proliferation. Groucho protein acts as corepressor to Wnt target genes by binding to and inhibiting TCF/LEF in the absence of  $\beta$ -catenin [14].

#### 1.1.1.3 Insulin like growth factor signaling pathway

Insulin-like growth factor signaling pathway has highly conserved function in mammals and plays a critical role in metabolism and cell renewal [15]. IGF pathway is not only involved in cell growth but it also, promotes cell proliferation, migration and malignant transformation [16]. IGF pathway includes four essential components which are: ligands, receptors, substrates and ligand binding proteins [17].

#### 1.1.1.4 The components of IGF signaling pathway

##### 1.1.1.4.1 A- IGF ligands

IGF ligands include both IGF-1 and IGF-2. Their names are based on the observation that both

IGF-1 and IGF-2 are peptides that have structure similarity to insulin and they share 40% homology with proinsulin [18]. However they are slightly different from insulin structurally by containing an additional domain which could account for their dramatically different role in neoplasms in comparison with insulin [19].

##### 1.1.1.4.2 B- IGF receptor

IGF ligands bind to the second component of the IGF axis which is the receptors that includes IGF-1 receptor (IGF-1R), IGF-2R, insulin receptor and hybrid receptors. The hybrid receptors consist of IGF-1R hemireceptor and insulin hemireceptor. IGF-1 and IGF-2 ligands bind to IGF-1R with high affinity while IGF-2 is the only ligand for IGF-2R [15] as shown in Fig. 3.

##### 1.1.1.4.3 C- substrates

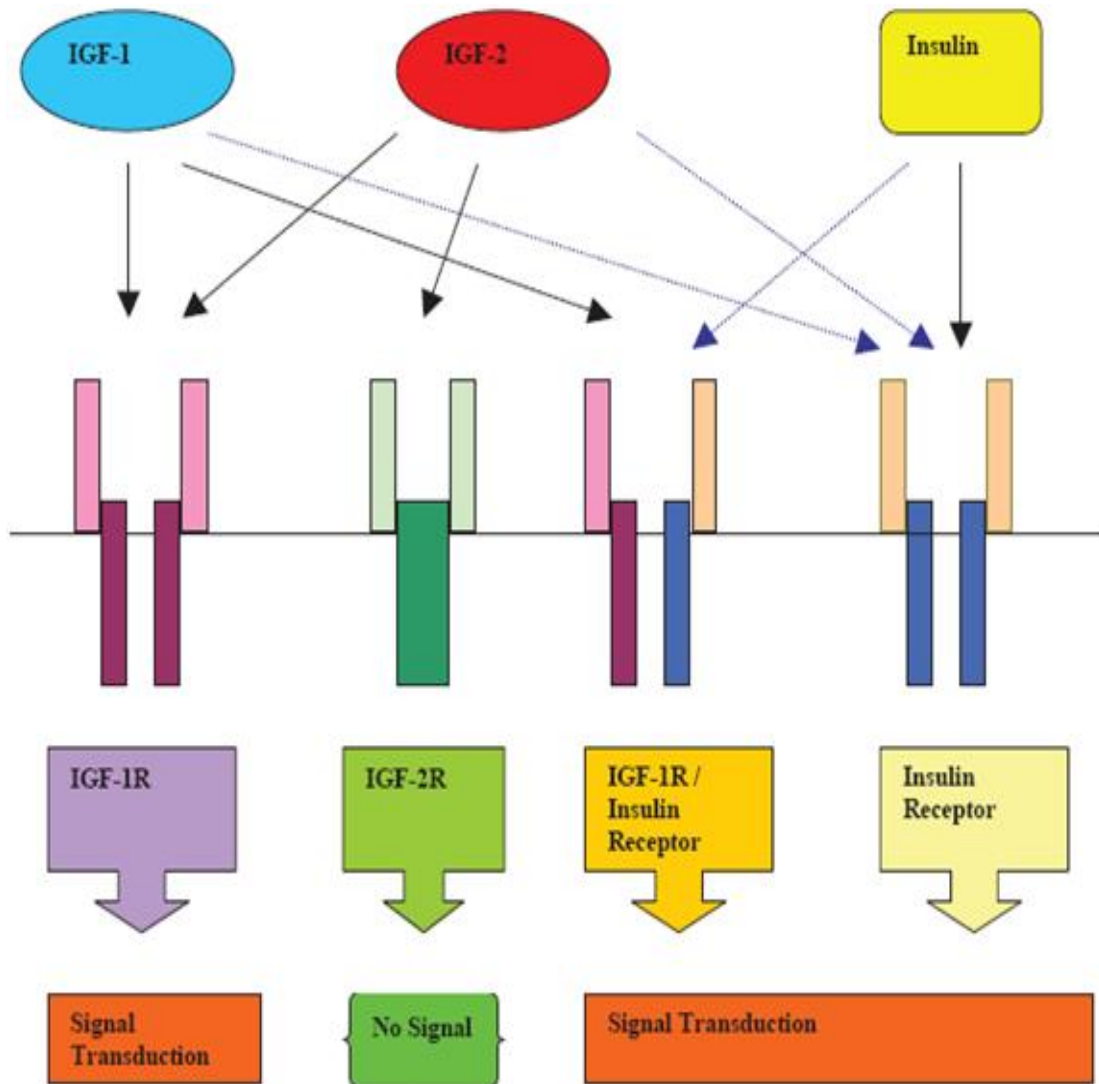
The third component of the IGF axis refers to insulin receptor substrate (IRS) proteins mainly, which are the major signals downstream of IGF-1R activation. There are 4 types of IRS and the important ones among them include IRS-1 and IRS-2 [19].

1.1.1.4.4 C- ligand binding proteins

The last key component of the IGF axis consists of IGF binding proteins (IGFBPs). There are 6 members of IGFBPs with high affinities for IGF-1 and IGF-2. For example, IGFBPs1-4 bind both IGF-1 and IGF-2 with similar affinity but IGFBP-5 and 6 strongly prefer IGF-2 as their ligand [17].

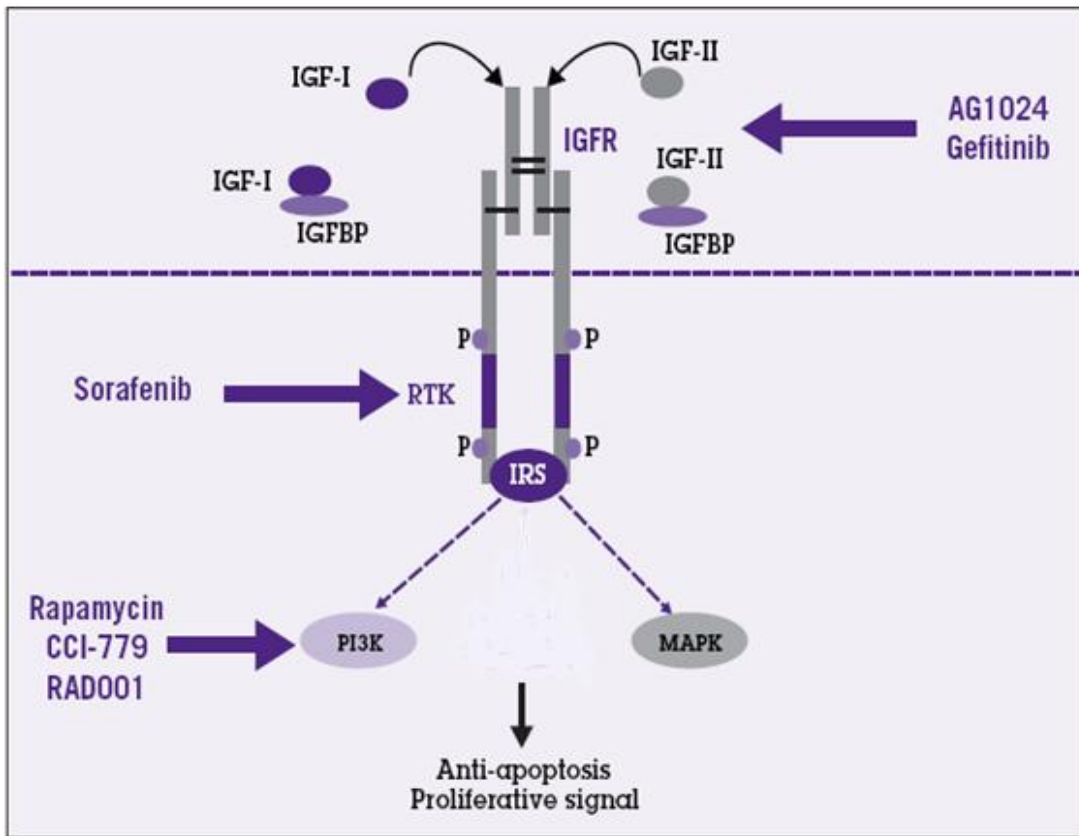
IGF signaling is initiated by the binding of IGF ligand to the receptor (IGF-1R), which

results in phosphorylation of intracellular portion of the receptors. Then, the phosphorylated receptor activates intracellular target proteins as IRS (by phosphorylation). IRS conveys the signal to specific downstream effectors such as phosphatidylinositol 3-kinase (PI3K) and MAPK pathways. The result of activation of these pathways is transcriptional activation of various target genes such as p27, cyclin B and VEGF gene [20] (Fig. 4).



**Fig. 3. Different types of IGF ligands and their receptors**

*Insulin receptor and IGF-1 receptor are both tyrosine kinases. IGF-2R functions as a clearance site for IGF-2. IGF-1 binds to IGF-1R and to IGF-1R/insulin receptor hemireceptor; it binds to insulin receptor only at very high concentrations. IGF-2 binds to IGF-1R, IGF-2R and binds to insulin receptor only during early fetal development. Insulin binds to insulin receptor and it binds to IGF-1R/Insulin hemireceptor at high concentration of the insulin. Signal transduction is activated after the activation of IGF-1R, IGF-1R/Insulin receptor hemireceptor and insulin receptor; however, IGF-2R activation results in no signal downstream. (Quoted from Wu and Zhu, [17])*



**Fig. 4. Simplified schematic presentation of IGF signaling pathway**

*IGF-I and IGF-II bind to IGF1R which is RTK with high affinity resulting in phosphorylation of intracellular proteins including IRS. The signal is then conveyed to specific downstream effectors such as PI3K and MAPK pathways. These pathways play crucial roles in antiapoptosis as well as cell proliferation. Therapeutic agents such as AG1024 and gefitinib aim to block this signaling pathway at the receptor level and downstream targeting agents such as rapamycin, CCI-779 and RAD001 are possible agents for HCC treatment. (RTK: receptor tyrosine kinase) (Quoted from Kitisn et al., [8])*

#### 1.1.1.5 Toll like receptor signaling pathway

TLR signaling is the first line of protection against microbial pathogens as TLR represents one of the important pathways that mediate the innate immunity. TLR can discriminate between a variety of pathogens through the function of pattern-recognition of the receptors and these receptors recognize certain microbial components known as pathogen associated molecular patterns [21].

Thirteen mammalian TLRs have been described and 10 are expressed in humans and each one of the receptors is responsible for recognizing distinct bacterial, viral or fungal structures as shown in Fig. 5 [22].

TLRs are characterized by two conserved regions: the extracellular leucine-rich region and

the cytoplasmic Toll/IL-1 receptor (TIR) domain. TLRs share the initial common activation pathway mediated by ligand recognition by the extracellular domain of the receptor then activation of the TIR domain [23].

After TLRs activation, two signaling pathways mainly exist; the first is through the adaptor protein called myeloid differentiation factor 88 (MyD88) which is common to all TLRs and the other one is MyD88-independent pathway that is particular to the TLR3 and TLR4 signaling pathways [24]

#### 1.1.1.6 MyD88-dependent TLR signaling pathway

Upon stimulation, specific ligand binding to the receptor, of the signaling pathway conformational changes occur to TIR domain of the receptor



leading to recruitment of the MyD88 adaptor protein [25]. MyD88 recruits IL-1 receptor associated kinase 1 and 4 (IRAK1, 4). They associate with MyD88 leading to their activation then the activated IRAK associates with tumor necrosis factor receptor associated factor 6 (TRAF6) [24].

TRAF6 is recruited to the receptor complex and activated by IRAK-1 then TRAF6 dissociates from the receptor and associates with TGF- $\beta$  activated kinase1 (TAK1) and TAK1-binding proteins (TAB) TAB1 and TAB2. The complex of TRAF6, TAK1, TAB1 and TAB2 moves into the cytoplasm [26].

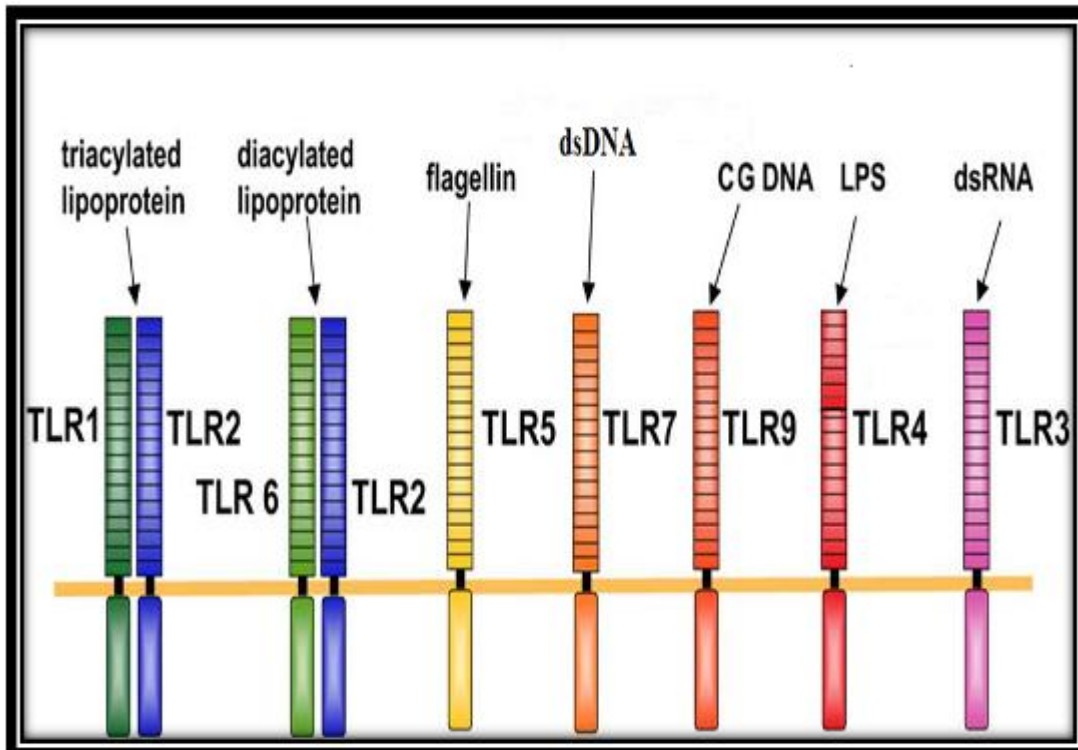
The activated complex (TAK1, TRAF6, TAB1 and TAB2) activates the inhibitory kappa kinase complex (IKK), which consists of 2 catalytic subunit (IKK $\alpha$ , IKK $\beta$ ) and one regulatory subunit (IKK $\gamma$ ), by phosphorylation at IKK $\beta$  subunit then the activated IKK phosphorylates the inhibitor of

NF- $\kappa$ B (I $\kappa$ B) leading to degradation of I $\kappa$ B and thereby releases the inhibition on NF- $\kappa$ B [27] as shown in Fig. 6.

#### 1.1.1.7 MyD88-independent TLR signaling pathway

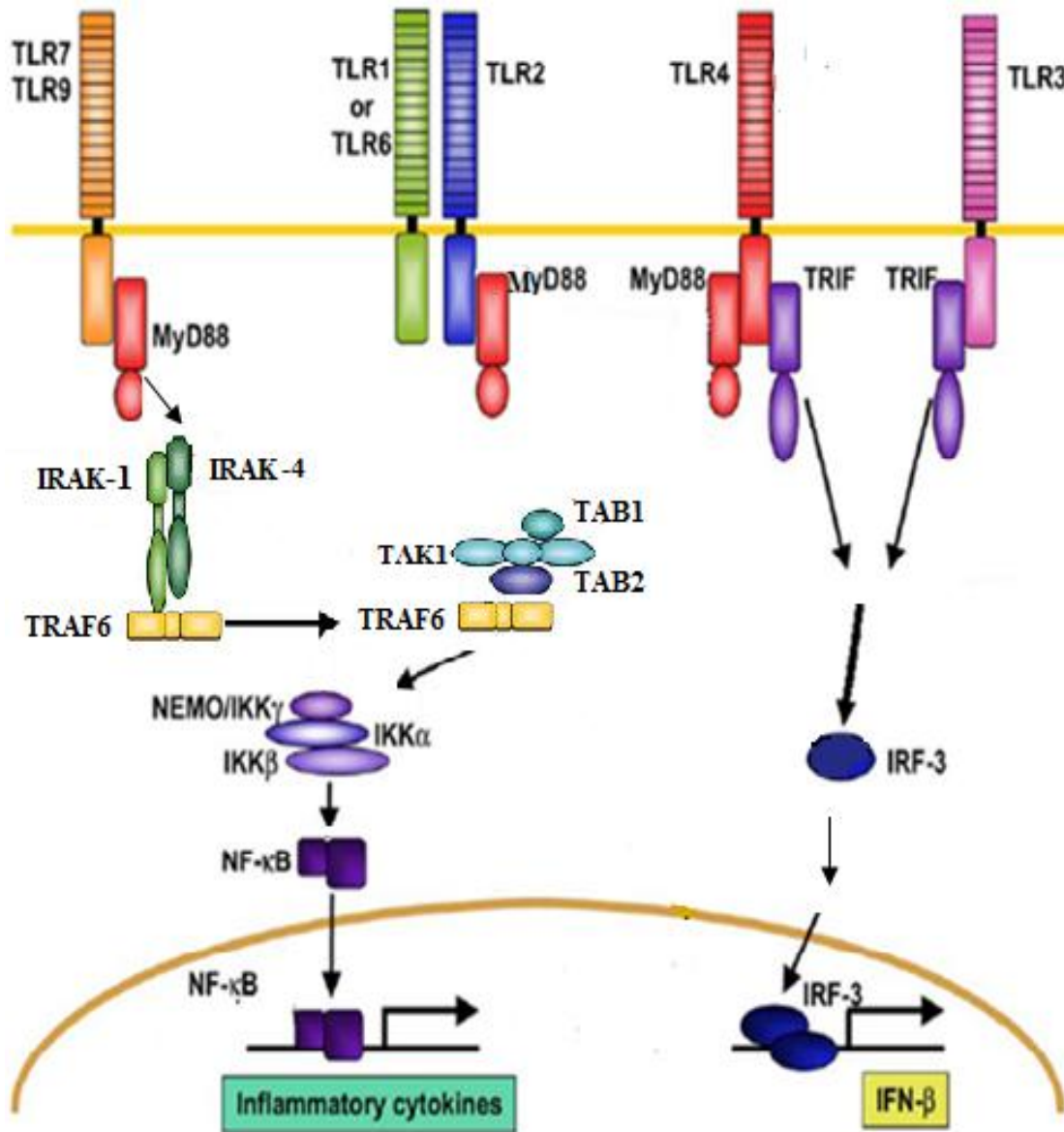
TLR4 and TLR3 have been shown to induce activation of the signaling pathway in MyD88 knockout animals through activation of another pathway called MyD88 independent pathway [28].

This pathway is mediated by another adaptor protein called TIR domain containing adaptor protein inducing interferon-beta (TRIF) that activates another downstream protein called interferon regulatory factors (IRFs) this protein passes to the nucleus and activates the gene coding for interferon-beta (IFN- $\beta$ ) leading to increased production of IFN- $\beta$  that mediate the body antiviral response against viral pathogens [29] as shown in Fig. 6.



**Fig. 5. Toll like receptors and the specific ligand for each receptor**

*TLR1–TLR9 have been characterized to recognize microbial components. TLR2 is present in the form of dimer with either TLR1 or TLR6 and discriminate the differences between triacyl and diacyl lipopeptides respectively. TLR4 recognizes bacterial LPS. TLR9 is the receptor for pathogen CG sequence in DNA, whereas TLR3 is implicated in the recognition of viral dsRNA. TLR5 is a receptor for bacterial flagellin. Thus, the TLR family discriminates between specific patterns of microbial components. (LPS: lipopolysaccharide, dsRNA= double stranded RNA) (Quoted from Takeda and Akira, [22]).*



**Fig. 6. MyD88 dependant and independent TLR signaling pathway**  
(Quoted from Takeda and Akira, [22])

#### 1.1.1.8 Notch signaling pathway

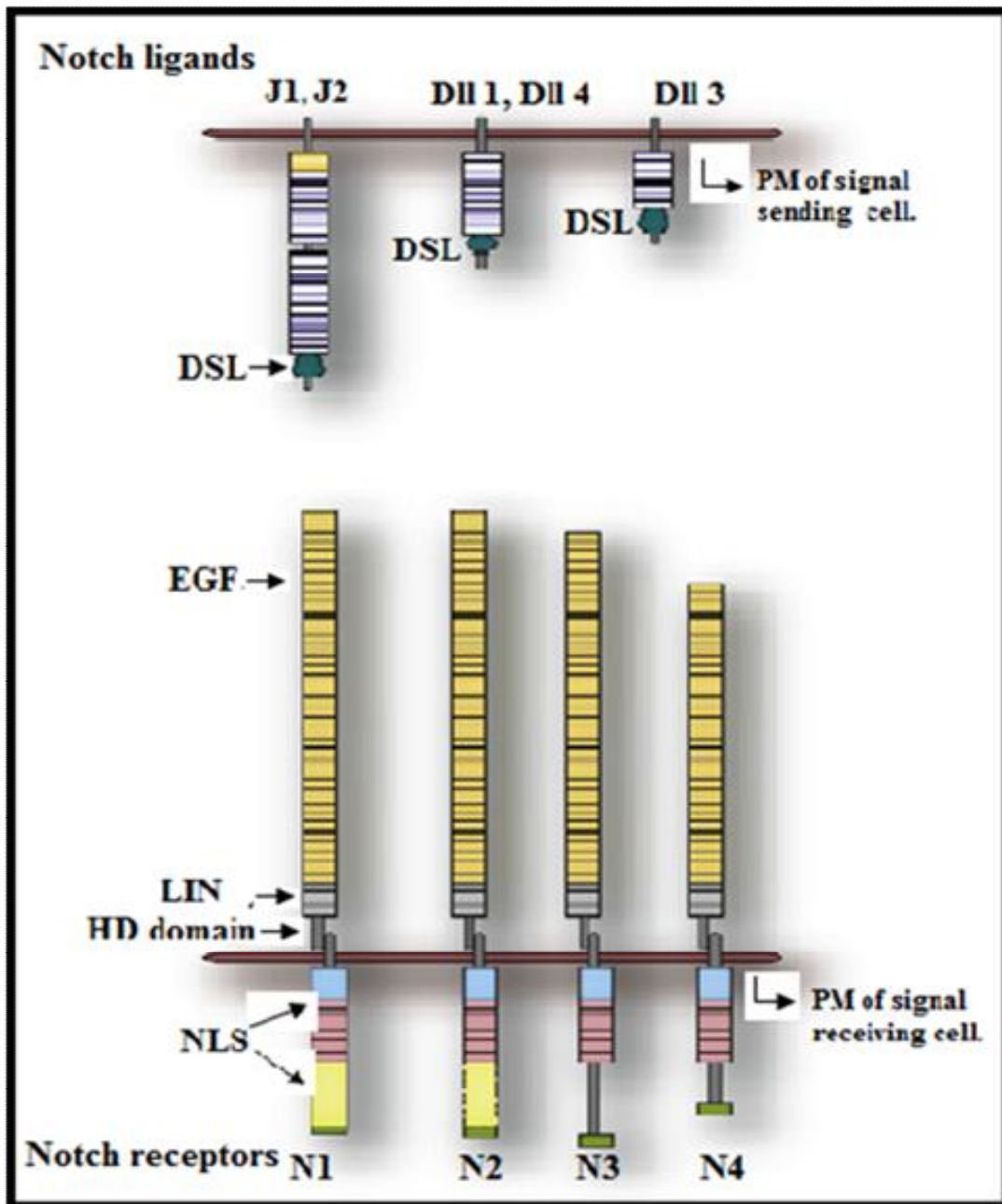
Notch signaling pathway has a role in maintaining the balance between cell proliferation, differentiation and apoptosis [30]. Mammals possess four Notch receptors (N) from N1 to N4. These receptors are present on the surface of the receptor cells and can bind to five ligands present on the surface of the signal sending cells and these ligands are named as Delta-like1, 3, 4 (Dll1, 3 and 4), Jagged 1 and 2 (J1 and J2) [ 31] as shown in Fig. 7.

The extracellular part of the receptors contains EGF like repeats involved in ligand binding, followed by three cysteine-rich repeats that prevent ligand independent activation (LIN) and a hydrophobic stretch of amino acids mediating heterodimerization (HD) between the notch receptor extracellular subunit (N<sup>EC</sup>) and the Notch receptor transmembrane subunit (N<sup>TM</sup>). The cytoplasmic tail of the receptor harbors nuclear localization signals domain (NLS) [32].

Newly synthesized Notch receptors are proteolytically cleaved in the Golgi at site 1



(S1) by a furin-like protease during noncovalently linked to a second subunit receptors transport to the cell surface. This containing both the transmembrane domain cleavage generates a heterodimeric receptor consisting of an extracellular subunit that is and the cytoplasmic region of the Notch receptor [34].



**Fig. 7. The structure of Notch ligands and their receptors**

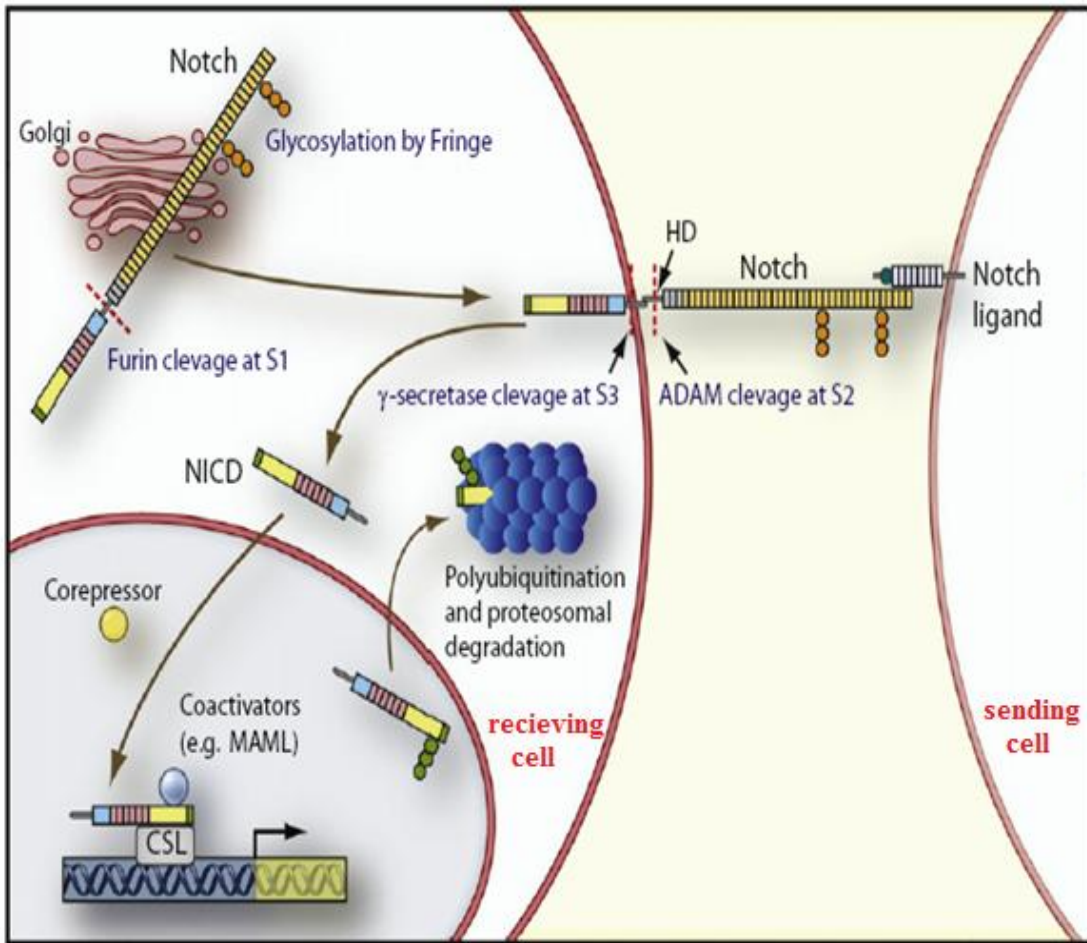
Five conventional Notch ligands are known: J1, J2, Dll1, Dll3, and Dll4. A common structural feature of all ligands is an amino-terminal domain called DSL involved in receptor binding and there is structural similarity between J1 and J2 and between Dll1 and Dll4. There are four Notch receptors (N1–N4) and these receptors possess extracellular, transmembrane and intracellular domains. (DSL: Delta, Serrate and Lag-2)  
(Quoted from Radtke et al. [33])

Notch signaling is activated upon cell to cell contact as a result of interactions between Notch receptors on the signal receiving cell and their ligands on the signal sending cell [31]. Ligand receptor interaction between neighboring cells leads to two successive proteolytic cleavages of the receptor. The first cleavage is mediated by a disintegrin and metalloproteases (ADAM) which cleaves the receptors 12–13 amino acids external to the transmembrane domain at S2 site. The shedded extracellular domain is endocytosed by the ligand expressing cell [35].

Ligand binding to N<sup>EC</sup> induces a conformational change within the notch receptors to expose the S2 cleavage site for proteolysis. After shedding

of the extracellular domain, a second cleavage between intracellular and trans-membrane domain (at site S3) is mediated by the  $\gamma$ -secretase activity [36].

The third cleavage liberates the intracellular domain of Notch receptors which subsequently traffics to the nucleus and heterodimerizes with core binding factor-1 (CBF-1) which is DNA transcription factor in order to form a short-lived nuclear transcription complex; then Notch intracellular domain recruits other coactivators including mastermind like proteins (MAML1-3) in order to induce the transcription of downstream target genes (Fig. 8) [33].



**Fig. 8. Notch signaling pathway**

*Notch proteins are synthesized as single precursor proteins, which are cleaved in the Golgi at site S1 and EGF-like repeats are glycosylated by fringe proteins in the Golgi before receptors are transported to the cell surface. The second cleavage occurs at site S2 followed by a third cleavage at S3. Notch intracellular domain undergoes polyubiquitination and proteosomal degradation in the cytoplasm after finishing its action as a transcription co-activator. (NICD: Notch intracellular domain) (Quoted from Radtke et al., [33])*

Notch target genes includes the hairy and enhancer of split 1 (Hes1), Hes5, Hes7 as well as Hes1-relate with YRPW motif (Hey1) and Hey2 [37]. Other Notch target genes are cyclin D1 and p21 gene. So notch signaling pathway is involved in the control of cell cycle, proliferation and apoptosis through its target genes [38].

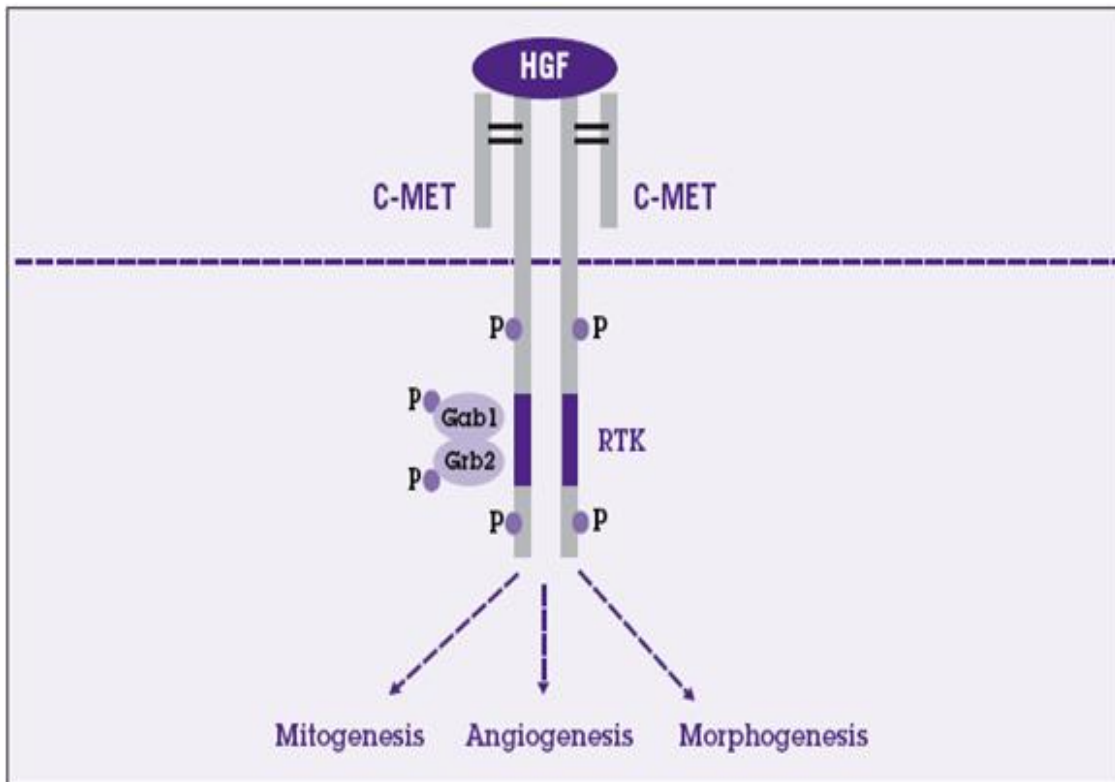
There are emerging data suggesting that Notch can crosstalk or cooperate with other signaling pathways including NF- $\kappa$ B or TGF- $\beta$  signaling pathway and thereby broaden the spectrum of target genes that are influenced by Notch signaling [39].

#### 1.1.1.9 Hepatocyte growth factor signaling pathway

Hepatocyte growth factor is one of the most potent growth factors for hepatocytes and plays a role in tissue proliferation, migration, cell survival, angiogenesis and tissue regeneration [40].

Hepatocyte growth factor binds to the receptor tyrosine kinase called c-MET, this receptor is highly expressed in epithelial and endothelial cells. Binding of HGF to the c-MET receptor results in receptor autophosphorylation then phosphorylation of adaptor proteins such as growth factor receptor bound protein 2 (Grb2) and Grb2 associated binding protein 1 (Gab1). The HGF signaling is then conveyed to activation of various downstream effectors such as phospholipase C and PI3K as shown in Fig. 9 [2].

Activation of the effectors leads to subsequent activation of distinct transcription factors as activator protein 1 leading to enhancement of the expression of numerous target genes as matrix metalloprotease and urokinase plasminogen activator genes. The main function of both urokinase plasminogen activator and matrix metalloprotease is extracellular matrix degradation. So, HGF has a role in increasing tumor invasiveness [41].



**Fig. 9. Simplified schematic presentation of HGF signaling pathway**

*Binding of the HGF to the extracellular domain of the c-MET receptor leads to autophosphorylation of the intracellular part of the receptor then activation of adaptor proteins (Grb2 and Gab1). Activation of these adaptor proteins leads to activation of various downstream effectors resulting in transcription of various genes important in mitogenesis, angiogenesis and morphogenesis (Quoted from Kitisin et al. [2])*

1.1.1.10 Transforming growth factor- $\alpha$ / Epidermal growth factor signaling pathway

TGF- $\alpha$ /EGF signaling comprises at least eight ligands including TGF- $\alpha$ , EGF, heparin-binding EGF, amphiregulin, betacellulin, epiregulin, epigen and crypto [42]. TGF- $\alpha$ /EGF signaling conveys its signal through the receptor tyrosine kinase Family which has four different types and EGFR is the most important one [43].

Different ligand specificities and concentrations lead to differential phosphorylation of tyrosine residues at the cytoplasmic portion of the receptor [44]. Binding of the TGF- $\alpha$  to the receptor causes phosphorylation of the receptor's cytoplasmic portion that serves as a docking site for some proteins such as sarcoma homology 2 domains protein (Shc) and Grb2 [45].

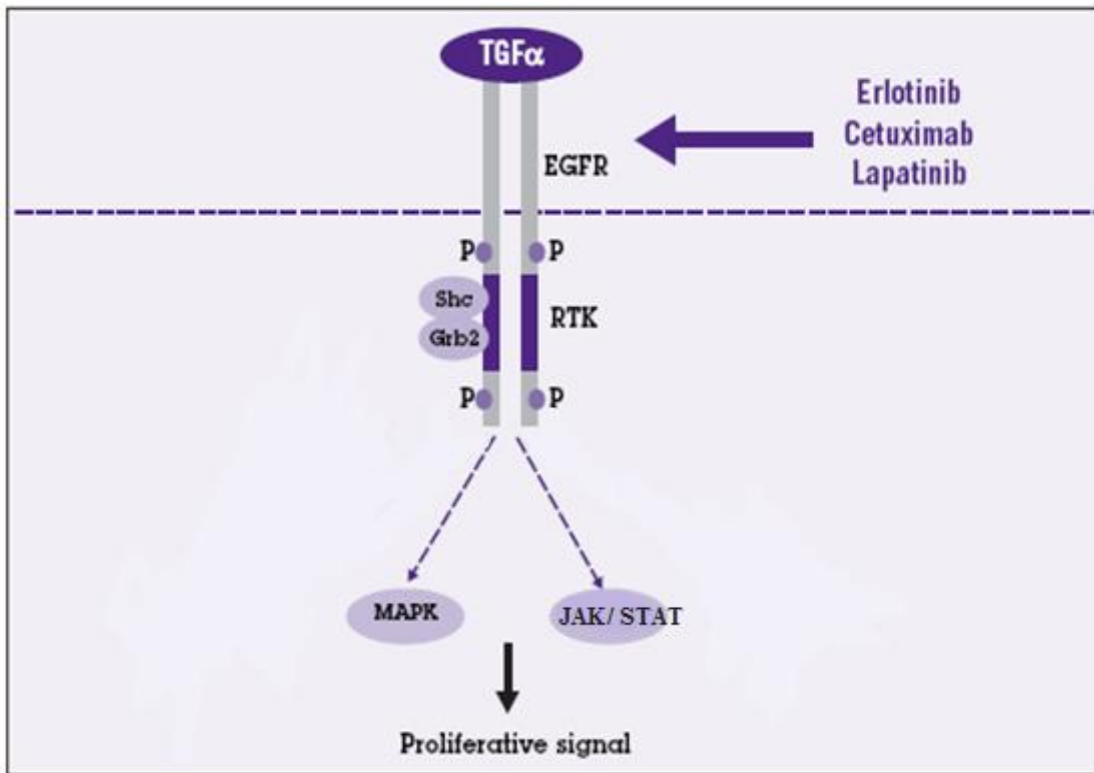
The signal will then activate multiple downstream pathways which can directly or indirectly interact with each other such as MAPK and JAK / STAT pathway [46] (Fig. 10).

1.1.1.11 Hedgehog (Hh) signaling pathway

Hedgehog signaling pathway is a complex network of signaling molecules including both positive and negative regulatory proteins which plays a crucial role in cell differentiation and proliferation [47].

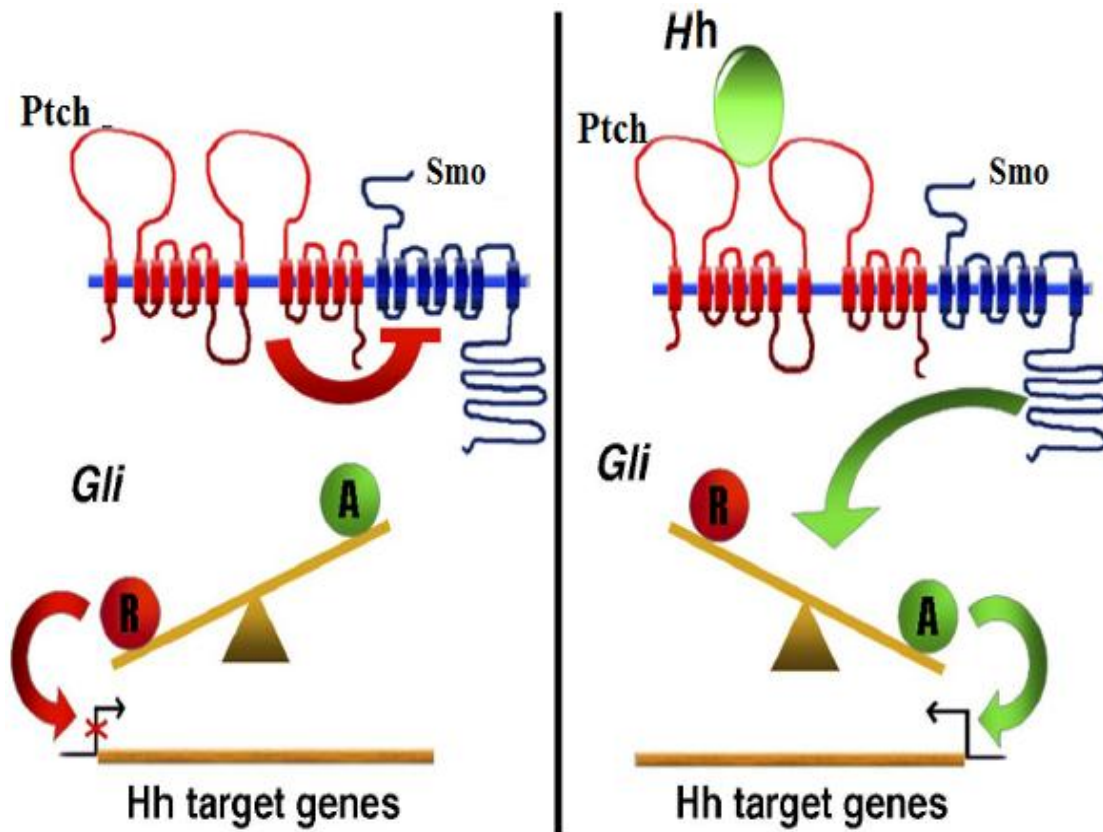
In the absence of Hh ligand, the membranous patched receptor (Ptch) antagonizes the pathway by preventing the activity of another transmembrane protein called smoothened (Smo) which is a 7-transmembranous protein receptor [48].

Binding of Hh ligands to Ptch relieves its inhibition on Smo. Smoothened protein activates some transcription factors called glioma-associated oncogenes (Gli-1, Gli-2, Gli-3). Different Gli proteins exhibit activating or repressing transcriptional activity. Gli-1 and Gli-2 mainly act as transcriptional activators while Gli-3 generates a repressor form in the absence or inhibition of Hh signaling as shown in Fig. 11 [49].



**Fig. 10. Schematic presentation of TGF- $\alpha$ /EGF signaling pathway**

*New therapeutic agents for HCC such as erlotinib, cetuximab and lapatinib are aiming to inhibit HCC growth and metastasis formation by acting as tyrosine kinase receptor inhibitors (Quoted from Kitisin et al. [2])*



**Fig. 11. Simplified presentation of hedgehog signaling pathway**

*In unstimulated cells (left), the activity of the transmembrane protein Smo is suppressed by the Ptch receptor and the majority of the Gli present is the transcription repressive (R). In stimulated cells (right) binding of Hh to Ptch activates Smo which in turn increase the level of the Gli transcription activator (A) resulting in the transcription of Hh target genes (Quoted from Parkin and Ingham, [49])*

Like  $\beta$ -catenin, after ligand stimulation, Gli accumulates in the nucleus and induces the expression of numerous target genes that regulate proliferation, differentiation and extracellular matrix (ECM) interactions such as fibroblast growth factor, IGF2, EGF, Cyclin B, Cyclin D and Cyclin E genes [50].

### **1.1.2 Signaling pathways associated with other types of cancer**

Each one of the incoming pathways is related to one or more of the previously mentioned liver stem cell signaling pathways.

#### **1.1.2.1 NF- $\kappa$ B signaling pathway**

NF- $\kappa$ B is a transcription factor that is considered to be a key regulator of the immune responses, inflammation and cell survival. Binding of the ligand to the TLRs is considered as a

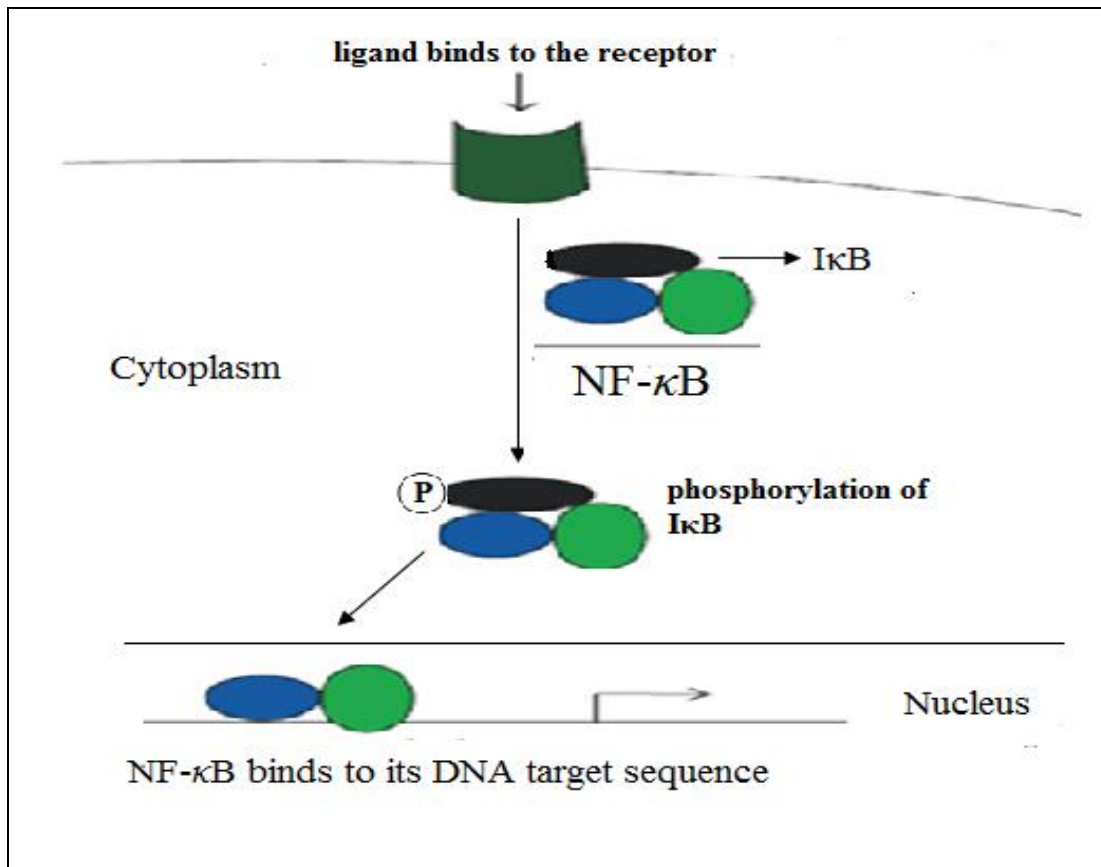
proinflammatory stimuli that activate NF- $\kappa$ B signaling mainly via IKK dependent phosphorylation then degradation of the phosphorylated I $\kappa$ B protein [51].

Once the inhibition on NF- $\kappa$ B is removed, it passes to the nucleus as shown in Fig. (12) and stimulates the transcription of genes encoding cytokines and antiapoptotic factors as genes coding for tumor necrosis factor-alpha, IL-1, IL-6, IL-10, IL-12 and many different chemokines [52].

#### **1.1.2.2 MAPK pathway**

MAPK pathway is a chain of proteins in the cell that communicates a signal from receptor tyrosine kinase (for example, EGFR) on the surface of the cell to the DNA in the nucleus of the cell. Thus, MAPK is a signaling component that is important in converting extracellular stimuli into a wide range of cellular responses [5].





**Fig. 12. NF- κB signaling pathway**  
(Quoted from Maeda, [52])

The signal starts when a signaling molecule binds to the receptor on the cell surface leading to MAPK activation by phosphorylation that is catalysed by MAPK kinases. After activation, MAPKs phosphorylate specific serine and threonine residues of target substrates, which include other protein kinases and many transcription factors affecting the expression of some proteins that controls cell cycle progression, apoptosis resistance and extracellular matrix remodeling [5].

#### 1.1.2.3 JAK/STAT pathway

JAK/STAT pathway plays an important role in cellular processes like differentiation, proliferation, apoptosis and cell adhesion. STAT is located in the cytoplasm and become activated through tyrosine phosphorylation which typically occurs through JAK or growth factor receptor tyrosine kinases such as EGFR [5].

Activated STAT enter the nucleus and serve as transcription factors as it acts as a regulator of

gene expression of various proteins including those for cell cycle progression, apoptosis resistance, extracellular matrix remodeling [5].

## 1.2 Cross-talk between Different Signaling Pathways Involved in HCC

Cytoplasmic downstream components of most growth factor signaling pathway complexes such as adaptor proteins, kinases and transcription factors are frequently utilized by more than one signaling pathway and numerous cross-talks between these pathways exist [43] for example:

### 1.2.1 Crosstalk between TGF-β and other signaling pathways

TGF-β signal is mediated through the Smad protein. The TGF-α/EGF as well as the HGF signaling causes phosphorylation of Smad2 potentially via some kinases leading to TGF-β signaling activation [5].

**1.2.2 Crosstalk between Wnt/  $\beta$ -catenin and other signaling pathways**

Dysregulation of Wnt signaling is traditionally attributed to mutations in Axin, APC and  $\beta$ -catenin genes that lead to constitutive hyperactivation of the pathway. However, Wnt/ $\beta$ -catenin signaling is also modulated through various other mechanisms in cancer, including cross talk with other signaling pathways [53-58] for example:

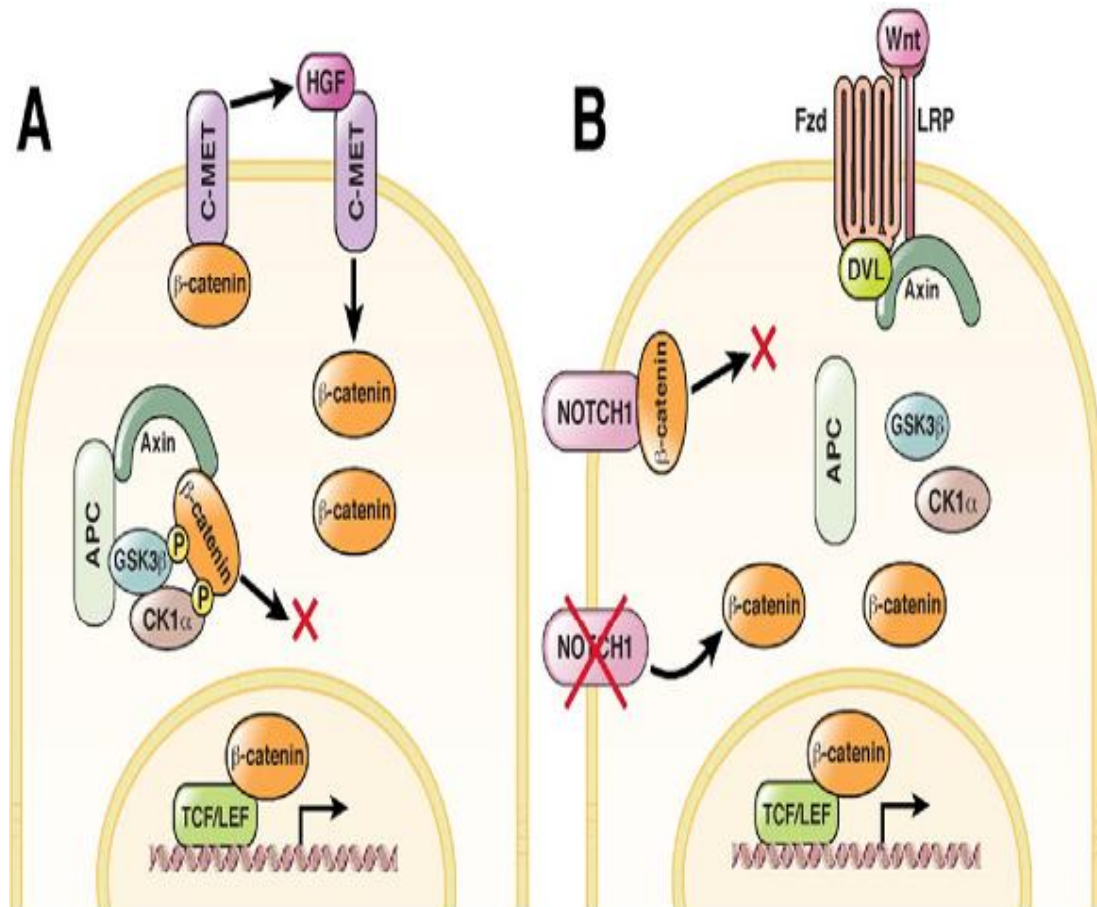
**1.2.2.1 Cross talk between Wnt and TGF- $\beta$  signaling pathway**

This important association involves Wnt and TGF- $\beta$  signaling pathways. Well-characterized association between  $\beta$ -catenin and E-cadherin is noted at the hepatocyte membrane and this

connection has significant implications in cell-cell adhesion [59] as shown in Fig. 13.

In HCC cells, TGF- $\beta$  signaling has been reported to correlate with the nuclear accumulation of  $\beta$ -catenin and may cause epithelial to mesenchymal transition (EMT). TGF- $\beta$  induces the expression of snail and slug, two transcription factors, that induces EMT via downregulation of E-cadherin gene which in turn causes the disruption of the cell-cell adhesion [60].

This event has been shown to inhibit the  $\beta$ -catenin and E-cadherin association at the hepatocyte membrane causing nuclear accumulation of  $\beta$ -catenin and stimulation of  $\beta$ -catenin target genes so this is considered as a connection between Wnt and TGF- $\beta$  signaling pathways [61].



**Fig. 13. Mechanisms by which Wnt/ $\beta$ -catenin signaling can be modulated in cancer. (A) In hepatocytes,  $\beta$ -catenin crosstalk with HGF pathway (B) Membrane-bound NOTCH1 can bind activated  $\beta$ -catenin and cause its lysosomal destruction**  
(Quoted from White et al. [13])

### 1.2.2.2 Cross talk between Wnt and HGF signaling pathway

In hepatocytes,  $\beta$ -catenin can be released from the membrane bound pool associated with the receptor c-MET. When c-MET engaged by HGF ligand, c-MET releases  $\beta$ -catenin into the cytoplasm with eventual  $\beta$ -catenin translocation into the nucleus and activation of its target genes as shown in Fig. 13 [13].

### 1.2.2.3 Cross talk between Wnt and Notch signaling pathway

The membrane bound Notch1 receptor can bind to the active free cytoplasmic  $\beta$ -catenin and negatively regulate it. Decrease in Notch1 expression may therefore potentiate Wnt/ $\beta$ -catenin signaling in certain types of cancer [62] as shown in Fig. 13.

## 2. DYSREGULATION OF SIGNALING PATHWAYS IN HCC

Dysregulation of different signaling pathways at any level including the receptors and downstream signaling pathway components represent a central protumorigenic principle in human hepatocarcinogenesis especially in liver stem cell signaling pathways [43].

### 2.1 Dysregulation of Signaling Pathways Associated with High Risk Groups for HCC

Exposure to risk factors for HCC highly predisposes to dysregulation in multiple signaling pathways including:

#### 2.1.1 Transforming growth factor-beta signaling pathway dysregulation

There is marked attenuation of the TGF- $\beta$  mediated antiproliferative response which has been shown in human HCC as there is marked reduction of TGF- $\beta$  receptors on the surface of the hepatocytes in up to 70% of HCC patients [43].

It has been reported that Smad proteins play a role in HCC as Smad4 is mutated in 10% of HCC patients; also Smad2 mutations are identified in about 5% of HCC patients. Finally, inhibitory Smad7 is upregulated in 60% of advanced HCC [63].

TGF- $\beta$  has antiproliferative role on the normal hepatocytes but in malignant hepatocytes, its

expression increase and enhance malignant hepatocytes proliferation. This dual role of TGF- $\beta$  signaling in HCC may be explained by its effect on the microenvironment of the tumor as tumor derived TGF- $\beta$  could contribute to tumor growth indirectly by suppressing immune surveillance or stimulating production of angiogenic factors [64].

TGF- $\beta$  signaling has also, been shown to induce an EMT in cells. This EMT process is characterized by decreased cell-cell adhesion through the decrease in E-cadherin protein production leading to enhanced migration and invasiveness [65].

Under normal conditions, TGF- $\beta$ 1 stimulates the proliferation of fibroblasts and induces the production of ECM protein as collagen in response to liver injury to repair the injured tissue. When liver injury is repeated, as occurs during persistent hepatitis virus infection (for example, HCV and HBV), the production of ECM continues and fibrous materials accumulate in the liver thus leading to cirrhosis that predisposes the liver to HCC as shown in Fig. 14 [66].

#### 2.1.2 Wnt / $\beta$ -catenin signaling pathway dysregulation

Alterations in Wnt signaling components have been described in HCC as up to 40% of HCCs exhibit accumulation of nuclear  $\beta$ -catenin [67]. Axin has been found to be mutationally inactivated in 3% to 14% of HCC cases [68] and the Fzd receptor is frequently overexpressed in HCC patients. These results suggest that more than one portion of the Wnt signaling pathway is dysregulated in HCC [69].

Studies implicate direct roles for hepatitis B virus and hepatitis C virus in modulating Wnt/  $\beta$ -catenin signaling. Hepatitis C virus core protein correlates with increased Wnt ligand expression in HCC and genes inhibitory to Wnt/ $\beta$ -catenin signaling are preferentially methylated in hepatitis C virus related HCC [70]. Hepatitis B virus X protein is able to bind APC and displaces  $\beta$ -catenin from the destruction complex, resulting in activation of Wnt/  $\beta$ -catenin signaling pathway [71].

#### 2.1.3 Insulin like growth factor signaling pathway dysregulation

Dysregulation of IGF signaling in HCC occurs predominantly at the level of IGF-2 ligand

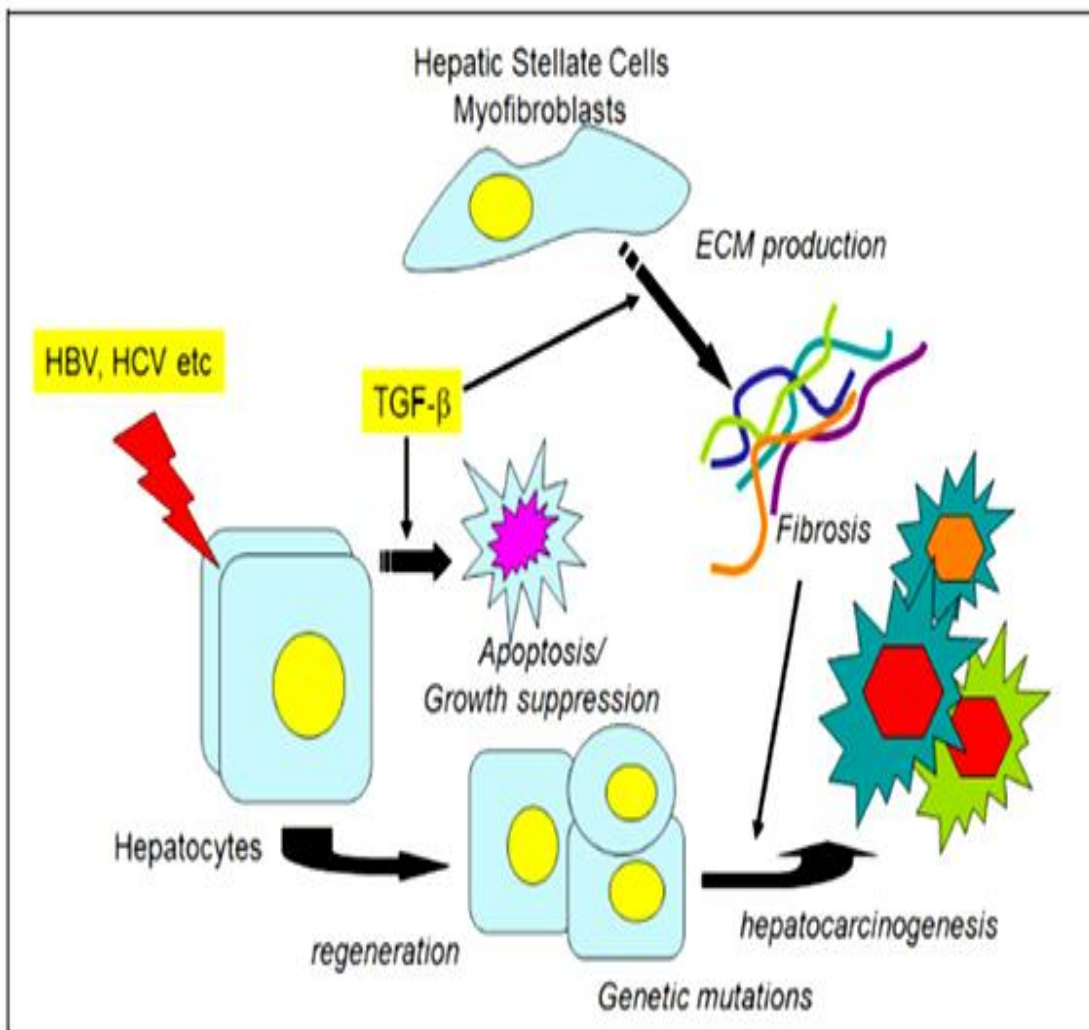
bioavailability. IGF-2 ligand bioavailability is inversely dependent on IGF-2R expression level [43]. A reduced IGF-2R level is associated with less ligand-receptor binding and thus, a relative increase in local IGF-2 bioavailability. IGF-2R expression level is reduced in 63% of HCC patients and mutation at IGF-2R gene has been implicated as a pre-neoplastic lesion for HCC [72].

In chronic hepatitis B infection, HBx protein contributes to overexpression of IGF-2 gene [73] and in chronic hepatitis C infection HCV

core protein also, induces overexpression of IGF-2 leading to induction of HCC development [74].

#### 2.1.4 TLR signaling pathway dysregulation

There is evidence that the synergism between alcohol abuse with hepatitis C and hepatitis B infection in inducing hepatocellular carcinoma is due to activation of a common pathway which is Toll like receptor signaling causing induction of proinflammatory cytokine production by NF- $\kappa$ B activation [75].



**Fig. 14. Transforming growth factor-beta, fibrosis and HCC**

*In the earlier stages of the liver injury, TGF- $\beta$  factor stimulates the proliferation of hepatocytes to restore the liver function or induces the apoptosis of injured hepatocytes to maintain tissue homeostasis. If the injurious agent persists TGF- $\beta$  stimulates ECM protein production from the activated hepatic stellate cells and myofibroblasts. The persistent inflammation, the accumulated ECM proteins along with the genetic mutations lead to the development of HCC (Quoted from Ozaki et al. [66])*

The HCV core and NS3 protein activate TLR2/TLR1 and TLR2/TLR6 leading to the production of inflammatory cytokines [28]. There is also marked upregulation of TLR2 and TLR4 detected in the hepatocytes and Kupffer cells in patients with chronic HCV. So hepatitis C virus infection contributes to the proinflammatory cytokine production by activation of TLR signaling pathway [76].

Alcohol ingestion decreases the intestinal mucosal barrier to LPS leading to the passage of the bacteria that has LP in its cell wall to the liver via the portal circulation. LPS leads to the activation of TLR4 on Kupffer cells causing pro-inflammatory cytokine production. Chronic alcohol consumption activates other TLRs, such as TLR1, 2 and 6-9 leading to further increase in the proinflammatory cytokines production [77].

### **2.1.5 Notch signaling pathway dysregulation**

Notch has been shown to act as an oncogene in human cancer [78]. When acting as an oncogene Notch1 receptor and the signaling pathway are significantly upregulated which results in increased cellular proliferation, prevention of differentiation, decrease immune attack against the malignant cells and inhibition of apoptosis [36].

HCV infection causes induction of Notch signaling pathway by causing damage to the liver cell and the damaged liver cells activates Notch signaling pathway to induce liver cell proliferation [79].

### **2.1.6 Hepatocyte growth factor signaling pathway dysregulation**

Patients in late stages of HCC had higher levels of serum HGF than healthy controls. So higher serum levels of HGF negatively correlate with patient survival time and positively correlate with tumor size [80].

c-MET receptor is overexpressed in HCC as compared with normal liver as c-MET is overexpressed in 20% to 48% of HCC patients [81]. This overexpression of c-MET could be partly due to growth factor dependent transcriptional activation of c-MET encoding gene [82].

Although, HGF has been shown to be overexpressed in the liver of HCC patients as compared to the normal liver but it is not

expressed by tumor cells themselves [83]. Instead tumor cell products induce stellate cells and myofibroblasts to secrete HGF then HGF in turn stimulates tumor cell invasiveness [84].

### **2.1.7 Transforming growth factor- $\alpha$ signaling pathway dysregulation**

TGF- $\alpha$  has been shown to be overexpressed in human HCC and TGF- $\alpha$  appears to act as potent mitogen for hepatocytes during the early stages of hepatocarcinogenesis and its level is correlated with tumor differentiation and proliferation [85]. It has been reported that elevated urinary level of TGF- $\alpha$  in patients suffering from HCC [86].

Interestingly, there are multiple links between chronic HBV infection and TGF- $\alpha$  signaling pathway. As the expression of TGF- $\alpha$  ligand in HCC patients correlate with the presence of viral polypeptides, HBs and HBc antigen, in the malignant liver cells and also, HBV-DNA induces TGF- $\alpha$  ligand expression [43].

### **2.1.8 Hedgehog signaling pathway dysregulation**

The role of Hh pathway in the growth of tumors can be classified according to how the pathway is activated and these mechanisms include: Firstly, loss of function mutations in the pathway inhibitory proteins such as Ptch. Secondly, gain of function mutations in the pathway positive regulators such as Smo. Lastly: overexpression of the Hh ligands leading to autocrine or paracrine activation of the pathway [87].

It has been reported that there is increased expression of the transcription factor Gli1, Hh receptor and Smo in HCC tumor samples compared to normal liver tissue. It has been demonstrated that blocking the Hh pathway using cyclopamine, a steroid alkaloid that inhibits Hh signaling by binding to and inhibiting Smo action causes inhibition of cell proliferation, increases apoptosis and represses Hh induced genes such as cyclin D1 expression so Hh pathway blocker can be used for HCC treatment [88].

## **2.2 The Aim of Studying Dysregulated Signaling Pathways in HCC**

Most HCC patients are diagnosed at a late stage so surgical treatment offers potential cure only for a minority of patients and therapeutic success



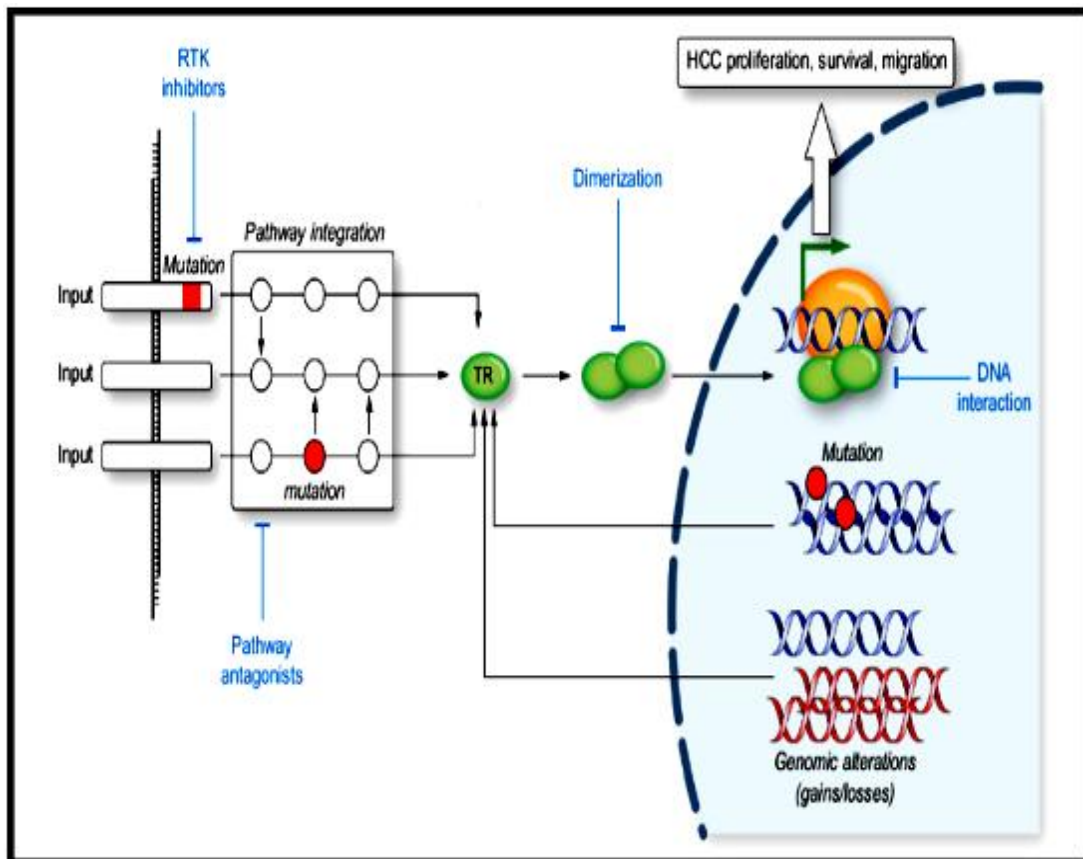
of pharmacological approaches as chemotherapy is limited [89]. So numerous experimental strategies are aimed to trigger signaling pathway dysregulation at different levels for a wide range of curative non-surgical treatment for HCC [2].

Interestingly, functional studies revealed that dysregulation of some signaling pathways as Wnt, IGF, TGF- $\beta$  and HGF signaling pathways may lead to HCC with specific clinical and biological features. Suggesting that receptor mediated signaling cascades may represent promising therapeutic targets [90].

Unfortunately, genetic heterogeneity of HCC severely complicates the development of effective and specific drugs which is reflected by the current lack of therapeutic options. For a

small number of patients, partial hepatic resection or liver transplantation offers potential cure and only the multi kinase inhibitor such as sorafenib has been established as the first effective and approved systemic treatment for advanced HCC [91].

However, a number of clinical trials have indicated that the administration of antibodies and kinase inhibitors targeting different tumor relevant receptors are potential therapeutic agents but they did not further improve the situation. For this reason, the identification of different levels of regulation of different signaling pathways in HCC would help to direct drug development and are of high relevance for improvement of the current dismal and unsatisfactory situation [92] as shown in Fig. 15.



**Fig. 15. Levels of dysregulation at signaling pathways and possibilities of interference in human HCC cells**

The Levels of dysregulation at signaling pathways (in red) include: a- Mutations of upstream signaling pathway components (e.g., receptors and pathway constituents). b- Mutations and genomic alterations in genes coding for transcriptional regulators (TR). Possibilities of pathway interference (in blue) include the inhibition of receptor tyrosine kinase inhibitors, expression of pathway antagonists, inhibitors of TR-dimerization and inhibitors of TR/DNA interaction (Quoted from Malz et al. [89])

Some methodological approaches utilize small organic molecules that specifically inhibit the interaction between transcription factors and their respective DNA binding domains such as  $\beta$ -catenin/TCF interaction [93]. Many of these approaches have already been tested in HCC models showing promising preclinical anti tumorigenic effects [94].

In addition, several transcription factors form dimers or multimers in order to recognize and bind their respective DNA binding sites as p53 protein. Disturbing these protein/protein interactions by specific substances may destabilize transcriptional complex assembly and thereby inhibit dysfunctional transcriptional activity [89].

Another possible approach is reconstitution of some tumor suppressor genes product such as p53 activity: A- If the cause of the defect is mutation at TP53 gene, small molecules that bind to the mutant p53 protein such as PRIMA-1 that restore its tumor suppressive function can be used [95]. B- When the cause of the defect is not TP53 mutation but is MDM2 overexpression, p53 reconstituted by substances that block MDM2 dependent degradation of p53 protein can be explored [96].

### 3. CONCLUSION

Hepatocellular carcinoma is the most common primary cancer of the liver and the incidence is increasing as HCC has risen to become the fifth commonest malignancy worldwide and the third leading cause of cancer related death. In addition, HCC carries a very bad prognosis as the 5-year survival rate is less than 5% in all HCC cases.

Hepatocarcinogenesis is a multistep process and this process may be induced by any of several risk factors that predisposes to HCC. Hepatocarcinogenesis may be mediated by multiple mechanisms but eventually all the mechanisms of HCC pathogenesis can create conditions that increases the chance of generating hepatocytes population containing critical combinations of structurally and functionally aberrant genes.

There are multiple risk factors for HCC and these risk factors can be classified into 1- Environmental risk factors such as chronic hepatitis B and hepatitis C virus 2- Metabolic risk factors such as diabetes mellitus and non-

alcoholic fatty liver disease 3- Hereditary risk factors such as haemochromatosis and others.

Some of the HCC risk factors could lead to dysregulation of important molecular cellular signaling pathways. This dysregulation may lead to malignant transformation by inducing alteration in many important genes – for example, genes coding for proteins that control the cell cycle, cell growth, inflammatory response and others.

There are multiple regulatory pathways dysregulated in HCC, for example: TGF- $\beta$  signaling pathway, Wnt/ $\beta$ -catenin signaling pathway, Toll like receptor signaling pathway, Notch signaling pathway, IGF signaling pathway, HGF signaling pathway and others.

The curative options for early stages of HCC are surgical resection followed by liver transplantation. However only 12% of patients are eligible for surgical intervention because most HCC patients are diagnosed at a late stage. So numerous experimental strategies are aiming to trigger the dysregulated signaling pathways in HCC for early diagnosis and a wide range of curative non surgical treatments.

Finally, it could be stated in concluding remarks that:

- 1) There are multiple mechanisms underlying the hepatocarcinogenesis and by understanding these mechanisms, early diagnosis for HCC could be possible.
- 2) Understanding the signaling pathways dysregulated in high risk groups for HCC may provide new diagnostic and therapeutic modalities.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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