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REVIEW

Targeting secret handshakes of biological processes for novel drug development

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Abstract **In multicellular organisms, several biological processes control the rise and fall of life. Different cell types communicate and co-operate in response to different stimulus through cell to cell signaling and regulate biologic processes in the cell/organism. Signaling in multicellular organism has to be made very secretly so that only the target cell responds to the signal. Of all the biomolecules, nature chose mainly proteins for secret delivery of information both inside and outside the cell. During cell signaling, proteins physically interact and shake hands for transfer of secret information by a phenomenon called as protein – protein interactions (PPIs). In both, extra and intracellular signaling processes PPIs play a crucial role. PPIs involved in cellular signaling are the primary cause for cell proliferation, differentiation, movement, metabolism, death and various other biological processes not mentioned here. These secret handshakes are very specific for specific functions. Any alterations/malfunctions in particular PPIs results in diseased condition. An overview of signaling pathways and importance of PPIs in cellular function and possibilities of targeting PPIs for novel drug development are discussed in this review.**

Keywords cell signaling, protein-protein interactions, peptide inhibitors

Introduction

In multicellular organisms, life begins by fusion of an egg with a sperm to form an embryo. Embryo formation is followed by transformation of a single cell embryo into a multicellular organism that involves cell division, production of various proteins, cell to cell communications, cell movement, cell death and various other complex biologic processes. Each cell in a multicellular organism is meant for a special function. The proteins expressed by a cell dictate the function of the cells and the protein composition is so important that by simple alteration of the composition in a cell, it can be made to survive or die. Majority of the proteins present in the cell do not function alone, they either interact with other proteins, DNA or RNA to execute its function. One important biological phenomenon that is essential, right from formation of embryo till the end of its life is PPIs. In multi cellular organisms, PPIs play an important role both at extra and intra cellular level for all biological processes. The importance of PPIs, identification of PPIs involved in various functions and how these PPIs can be targeted at different levels for development of novel drugs are discussed in this review.

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Different levels of PPIs

Extracellular interactions

At the extracellular level, the type of receptors expressed by a cell determines the type of ligand present in the environment with which it can interact and respond for the physiologic situation. These interactions include 1) Autocrine, wherein a cell produces its own growth factors and respond to it by binding to the extracellular receptor, 2) Paracrine, here the signaling molecules secreted by one cell, bind to the receptors present on the other cells in vicinity and regulates its function. These type of interactions play a major role during the embryo development 3) Juxtacrine, where two cells interact physically using the cell surface ligand and receptor to initiate a physiological process like the interaction between T cell and antigen presenting cells 4) Endocrine, in which special organs synthesize and secrete signaling molecules called as

hormones that act on cells present in a distant organ and regulate its function. In all these types of interactions, ligands bind to the receptors present on the surface of the cell using a specific domain. The amino acid sequences present in both the ligand and receptor determine the specificity of their binding (Hornák et al., 1999).

Intracellular interactions

Proteins are the important biomolecules which are produced by translation of genetic information stored in the nucleus in the form of DNA sequence. The physiology/function of a cell is determined by its protein composition. The changes in protein composition are brought about by alterations in the gene expression by a phenomenon called as differential gene expression. In a multicellular organism, changes in gene expression/function of a cell are mainly initiated by ligand – receptor interactions. While proteins and large polar ligands bind to cell surface membrane receptors and modulate expression of suitable genes, steroids and small hydrophobic molecules bind to receptors present in the cytoplasm and get activated, leading to changes in gene expression.

Irrespective of the type, the receptors on activation either themselves act as transcription factors (steroid receptors) or initiate a chain of reaction (cell surface receptors) known as signal transduction pathway which in turn activates specific transcription factors. The activated transcription factors translocate itself from cytoplasm to nucleus and bring about changes in gene expression (Secko, 2013). A cell possesses numerous types of surface membrane receptors and several signaling molecules in the cell. The challenge of a particular ligand is to generate a change in the expression of a set of genes in the nucleus by the phenomenon of PPI (Lodish et al., 2000).

Cell surface receptors usually contain three domains 1) extracellular domain 2) trans-membrane domain and 3) intracellular domain. The extracellular domain plays an important role in giving specificity for the ligand – receptor interaction, the role of trans-membrane domain is to anchor the receptors to the plasma membrane of the cell and the intracellular domain interacts with specific intracellular signaling proteins and plays an important role in initiating signaling pathway. In all the above mentioned processes PPIs plays an important role in binding of the ligand to its receptor and the production of required proteins.

Intranuclear interaction

Once the transcription factors are translocated inside the nucleus, these proteins bind to specific DNA sequences called as regulatory sequences. These sequences interact with various proteins involved in transcription regulation by creating a change in gene expression that leads to modification in protein composition and hence physiology of the cell. In the nucleus, two broad group of proteins exists apart from minimal proteins involved in transcribing machinery; nuclear co-activators and nuclear co-repressors. Of the two groups, depending on the type of proteins interacting with the transcribing machinery, the gene expression is either initiated or terminated at the vicinity of the newly arrived transcription factors (Server and Glass 2013).

In a multicellular organism, the cells are well defined to perform diverse functions. Though all the cells in an organism contain the same genome, over a period of development they acquire the ability to express specific set of genes and produce specific proteins. These specific proteins are required to perform their function, respond to various signals and synthesize various other proteins through differential gene expression, where PPIs play a very important role.

Post-translational modifications in cell signaling

Post-translational modifications (PTMs) of proteins and the domains that recognize these modifications play central role in signal transduction pathways that respond to alterations in the cellular microenvironment. PTMs are rapid and largely reversible. A protein's property is easily changed by the addition of simple chemical group such as PO_4^{2-} (phosphorylation), $-CH_3$ (methylation), $-CH_3CO^-$ (acetylation) etc. Addition of any of these small groups to one or more amino acids in a protein, changes the property of the protein greatly. Out of several PTMs known, phosphorylation plays a major role in cell signaling, a group of kinases and phosphatases mediate various cellular signaling by simple addition and removal of phosphate group on signaling molecules (Bononi et al., 2011). MAPK signaling pathway is the best example to understand the role of phosphorylation in mediating signal transduction pathway.

Extra cellular regulated kinase1 and 2 (ERK 1/2) belongs to MAPK group of proteins which are involved in ERK signaling. Binding of growth factors such as epidermal growth factor (EGF) to its receptor (EGFR) results in the activation of the cell proliferation. Upon ligand – receptor binding, intracellular domain of the receptor gets activated and leads to self phosphorylation of the intracellular domain on tyrosine residues. This modification on the cytoplasmic domain of EGFR attracts docking proteins such as GRB2 and SH2 domain. Assembly of docking proteins on EGFR domain attracts another protein called as SOS which activates Ras protein by exchanging GDP for GTP. GTP bound Ras is the active form of kinase and it initiates a cascade of events called as kinase cascade.

Ras in its active form specifically phosphorylates MAPKK at serine and threonine residues. Inactive MAPKK upon phosphorylation becomes an active kinase and it further activates ERK 1/2 by phosphorylating on Tyr 340/341 and Thr 491 amino acids. Phosphorylated ERK1/2 binds to specific transcription factors and activates them by phosphor-

ylating at Ser and threonine residues. The activated transcription factor finally brings about changes in the gene expression that are required for cell division (De Luca et al., 2012). In the whole cascade of phosphorylation events, PPI play a major role, the interactions and phosphorylation events are so specific that the whole cascade can be stopped by introducing mutations in one or more binding domains of the kinases or by simply mutating the Ser/Thr/Tyr residues involved in phosphorylation (Zhang and Liu, 2002).

Secret transfer of information in multicellular organism through PPIs

In a multicellular organism, where several types of cells are present, passage of information from one cell to another is so important and complex. The information needs to be passed to the target cells so secretly that only the intended cell responds to the signal while the others don't. While this is easily achieved by differential expression of cell surface receptors, so that the information is passed through ligands and specificity of the ligand receptor interaction plays a major role in secret transfer of information in the extra cellular region.

When it comes to cytoplasm, it becomes very complex, due to the presence of several hundred proteins in the cytoplasm most of which are involved in signaling. The information/signaling given at the surface of the cell need to be precisely and secretly transferred to the concerned signaling molecules without letting the other signaling molecules know; so that the expression of required genes for that particular stimulus is modulated in the nucleus by activation of specific transcription factors. This task in presence of several other signaling molecules is achieved by specificity in PPIs among the proteins involved in that particular signaling pathway. Considering the case of MAPKs there are mainly three different MAPKs present in the cell ERK, JNK and p38. Among these molecules, while ERK is involved mainly in cell proliferation, JNK in stress related signaling pathway and p38 in inflammatory related pathway. These molecules share a common mode of activation where the signals from the intracellular domain of the receptors are passed on to MAPK activation enzymes via MAPKKK to MAPKK to MAPK. Though there are several MAPKKs present in the cell, in response to mitogen stimulation only MAPKK 1/2 (MEK 1/2) are activated which specifically bind to and activate ERK 1/2 by phosphorylation. Though the MAPKK – MAPK interaction occurs using similar type of domain such as [- $(R/K)_2$ – $(X)_{2\text{-}6}$ – L/I – X- L/I-], the amino acid sequences in the interacting domains of both the proteins determines the specificity of these interactions. Hence inspite there are several MAPKKs present in the cell, Mitogen activated signaling is passed on only to MAPKK1/2 followed by ERK 1/2 (Enslenand Davis, 2001). Once ERK 1/2 are activated they interact with specific transcription factors required for transcription of genes necessary for cell division. These specificities in the interaction at the amino acid level can be demonstrated using mutant studies where a change in single amino acid in the interacting domains of any of the two molecules will prevent the interactions. (Enslen and Davis, 2001)

Negative regulators of signaling

It is clear that complex mechanisms exist in the cell to activate a particular signal transduction pathway. As a result of activation, changes in the gene expression are achieved in the cell in a way to respond to the stimulus. Once the required task for the pathway is achieved, it becomes essential to stop or reverse the reactions involved in the activation of signal transduction pathway.

T cell receptor (TCR) signaling is a good example to demonstrate the negative regulation of signaling. T cells are activated when TCR interacts with MHC molecules containing foreign peptides. TCR activation involves a series of phosphorylation reactions in the cytoplasmic proteins. However, once the T cells are activated it is essential to deactivate the signaling in order to avoid the hyper activation of the immune system. In these processes group of phosphatases play a dominant role. During TCR activation, cytoplasmic domain of the TCR acquires kinase activity and its tyrosine residues are phosphorylated. During negative regulation, specific dephosphorylation of activation loop sites in cytoplasmic domain of the TCR leads to inactivation of the kinase domain, while phosphate removal from tyrosines of docking proteins by specific phosphatases, blocks activation of specific signaling pathway. Several tyrosine phosphatases such as SHP2, Protein tyrosine phosphatases (PTP), MAPK phosphates (MKP1) play a dominant role in removing the phosphate group from the activated signaling molecules thereby returning them to inactive state. Even here the PPIs play a major role in determining the specificity in controlling a particular signaling pathway (Dikic and Giordano, 2003). Detailed study of negative regulators function helps us to design strategies to downregulate/upregulate a particular pathway and design drugs accordingly to modulate particular signal transduction pathway. LCK is one of the proximal signaling molecule involved in activation of TCR signaling, PEST domain enriched tyrosine phosphatase (PEP) specifically dephosphorylates p-LCK molecule and genetic deletion of PEP gene in mice lead to hyper activation of immune system (Hasegawa et al., 2004). This observation suggests that preventing phosphorylation of LCK molecule or inhibition of LCK activity is one of the strategies for suppression of immune system; in this regard research is on the way to develop inhibitors for LCK (Meyn and Smithgall, 2008).

Malformation in PPIs lead to diseases

To highlight the importance of PPIs and any malformation in these interactions lead to diseased condition, the following examples are discussed. Several human diseases are caused due to the abnormal protein production, which may lead to a loss either in the ability to interact with its partner or in forming a new protein complex. Genetic defects may also disturb the existing protein interactions. Some of the PPIs related diseases are discussed below.

Huntington's disease is one of the several inherited neurodegenerative disorders caused due to expansion of the polyglutamine (polyQ) proteins. These polyQ regions are responsible for the formation of abnormal Huntingtin protein aggregates in the brain resulting in cognitive defects, psychiatric abnormalities and movement disorders (Shi-Hua Li and Xiao-Jiang Li, 2004). The N-terminal region of the protein Huntingtin, a ubiquitously expressed protein in neurons, interacts with several other proteins over a wide range of functions such as anti-apoptotic effects, transcription regulation etc. In the case of diseased condition the proteins gets aggregated which results in loss of function of the protein due to a loss in PPIs required for normal function of the cell.

Purkinje cell degeneration in brain leads to several human neurodegenerative disorders such as spinocerebellar ataxias which lead to ataxia, or loss of balance and coordination. These are caused due to either loss or gain of function of proteins. Using both *in silico* and *in vivo* analysis, Lim et al., (2006) have reported that several hundred PPIs are involved in normal functioning of neuronal system and any alterations in these interactions lead to disease.

Gab family of proteins belongs to scaffolding adapters that are involved in signal transduction process. They are evoked by extracellular stimuli and play an important role in various physiological processes through association with other signaling molecules in cytoplasm. Certain allelic variants of Gab 2 protein lead to high risk of Alzheimer's disease (Reiman et al., 2007), these variants may differ in their interacting partners leading to defective signaling pathway responsible for pathogenesis of the disease. Malfunctioning in Gab 2 has also been associated with impaired allergic response (Gu et al., 2001), osteoporosis (Wada et al., 2005), and abnormal hematopoiesis (Zhang et al., 2007) and other human diseases (Nakaoka and Komuro, 2012)

Baller gerold syndrome, a rare congenital disease is caused due to replacement of isoleucine to valine due to mutation in H-Twist protein. This mutation is located at the interaction interface of the protein. There is an overlap in the phenotype of these patients with other patients having diseases such as Rothmund-Thomson syndrome or Saethre-Chotzen syndrome. Later it was identified that isoleucine to valine substitution at position 156 of the H-Twist protein as the causative mutation (Seto et al., 2001). By yeast – two – hybrid system it was reported that this substitution mutation resulted in loss of H-Twist protein to interact with E12 protein, demonstrating the importance of PPIs in normal physiology (El Ghouzzi et al., 2000). Adrenocorticotropin hormone deficiency leads to weight loss, anorexia, low blood pressure and other symptoms. T- box transcription factor TBX19 is essential for transcription of the gene encoding this hormone. A mutation in this transcription factor where Ser128 substituted to Phe leads to a dominant loss of function phenotype, leading to inability of the transcription factor binding to DNA and helping in transcription of the gene (Asteria, 2002). The above two examples show that every single amino acid involved in the PPIs are essential for normal functioning of a cell/organism and mutations altering the amino acid sequences of interaction domains lead to malfunctioning of signal transduction pathway.

PPIs play an important role in host-pathogen interaction also; most of the viral processes are the result of the coordinated protein interactions. Several of the viral enzymes are oligomeric proteins. The three enzymes of HIV, protease, integrase and reverse transcriptase are homodimers, important for viral replication. The disruption of these dimers or formation of heterodimers against them prevents formation of functional complexes (Coffin et al., 1997)

Methods to identify protein–protein interactions

PPIs being the main strategy involved in signal transduction pathway, methods are developed to identify these interactions during different cellular stimuli. Yeast two hybrid (Y2H) system (Young 1998) is one of the oldest methods used for this purpose in past. Recent development in this field of studies include, immunoprecipitation of a particular signaling protein from whole cell lysate and identification of the proteins co-precipitated along with the signaling molecule by using mass spectrometry (Blikstad and Ivarsson, 2015). Affinity purification technique is another alternative to identify PPIs. In this technique the signaling protein of interest is immobilized in a column and whole cell lysate is passed through the column, the proteins that interact with the signaling protein are retained in the column and rest of the proteins are eluted out. The retained proteins in the column are later eluted and identified using mass spectrometry. Surface Plasmon resonance (SPR) is another optical technique used for detecting the protein interactions. Binding of a mobile molecule (analyte) to a molecule immobilized on a thin metal film (ligand) changes the refractive index of the film. The angle of extinction of light, reflected after polarized light impinges upon the film is monitored which directly detects the mass (concentration) with no need for labeling the peptides (Berggård et al., 2007). The other various techniques for identifying the PPIs are by tandem affinity purification (TAP) tag (Rigaut et al., 1999) and Phasor time resolved fluorescence (Jameson et al., 2013) etc.

Developing small molecule drugs targeting protein–protein interactions

Ongoing discussion highlights the importance of PPIs and their role in regulation of several if not all biological processes in multicellular organism. In the studies involving mutant signaling molecules it has been very well demonstrated that normal functioning of any signal transduction pathway lies in the proper interactions between the proteins involved in that particular signaling. Therefore development of agents that can interfere in particular PPI can modulate/rectify defective signal transduction pathways responsible for a particular disease by working on a simple principle that any diseased condition is due to hyper activation or deactivation of particular PPIs. In the past, several drugs have been developed to interfere in the PPIs at various levels as shown in the Fig. 1.

1) Manipulation of extracellular ligand-receptor interactions: Several drugs have been developed that can either block the ligand –receptor interaction (antagonist) or drugs that can bind to the receptor with better affinity than the original ligand (agonist). Example: Gonadotropin releasing hormone (GnRH) is an important hormone secreted by pituitary for normal functioning of reproductive system. GnRH antagonists such as Ganirelix competitively and reversibly bind to GnRH receptors in the pituitary gland and block the release of luteinising hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary. In men, the reduction in LH subsequently leads to rapid suppression of testosterone release from the testes; in women it leads to suppression of estrogen release from the ovaries. Similarly the GnRH agonists such as buserelin, cause stimulation of the hypothalamic-pituitary-gonadal axis (HPGA), by binding to GnRH receptor with high affinity, leading to a surge in testosterone or estrogen levels (Filicori and Flamigni, 1988).

2) Targeting PPIs at cytoplasm: With reference to the cytoplasm, identification of particular signal transduction pathway involved in a physiologic process and drugs to inhibit or activate specific pathways have been achieved. JNK MAPK signaling in response to stress causes neurological disorders such as Parkinson's disease and ischemic stroke. Inhibitors of JNK upstream kinases were developed such as CEP-1347 which reduces the kinase activity of MLKs and thus inactivating the JNK pathway (Kuan and Burke, 2005). Similarly protein kinase C (PKC) is involved in variety of signal transduction pathway including cell proliferation. Mezerein binds to PKC instead of its natural activator Di Acyl glycerol (DAG). It has a higher affinity for PKC than DAG does, and it cannot be degraded as easily as DAG. Therefore, when mezerein is bound, PKC remains in the active conformation much longer than it normally does. Furthermore, when mezerein has bound to PKC, PKC no longer requires Ca^{2+} for activation. This causes overstimulation of the pathways initiated by PKC, leading to sustained activation of downstream signaling molecules, leading to more cellular proliferation (Black and Black, 2013)

3) Inhibiting nuclear translocalization of transcription factors: The hallmark of signal transduction pathway is nuclear translocalization of specific transcription factors by PTMs. Each transcription factors interact with their upstream kinases for activation by PTMs. Inhibition of specific kinases prevent activation of specific transcription factors and thus preventing the expression of those genes that are supposed to be activated by the transcription factors. Interaction and phosphorylation of c Jun transcription factor by JNK MAPK

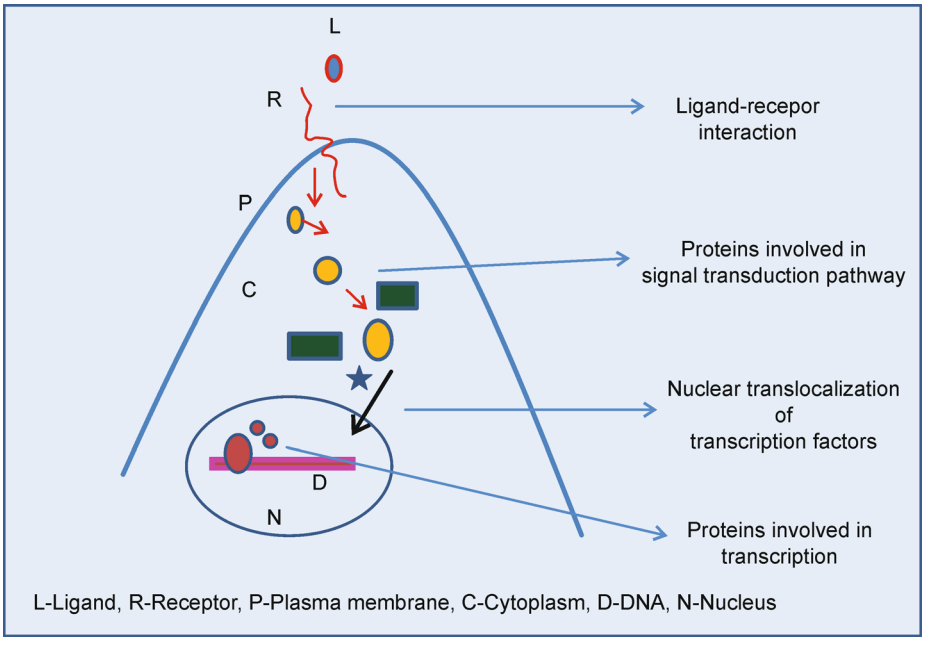

Figure 1 Different sites where protein-protein interactions can be targeted.

is essential for its activation. Several JNK inhibitors have been developed that prevent activation of c Jun and hence changes in physiological activities mediated by c Jun; such as apoptosis (Table 1).

4) Targeting PPIs in the nucleus: The interacting partners of the activated transcription factor bound to DNA sequences determine whether the gene in the vicinity will be transcribed or not. Drugs have been developed to manipulate PPIs in the nucleus and manipulate genes expressed by transcription factors; a good example in this class of drug is SERMs (Selective estrogen receptors modulators). SERMs are a class of drugs that act on the estrogen receptor (ER), these drugs are different from pure ER agonists and antagonists. They have different effect on different tissues, with respect to estrogen action. These drugs selectively interact with different nuclear co-regulators and regulate expression of estrogen regulated genes in tissue specific manner. SERMs provide estrogenic benefit to the cardiovascular and skeletal systems, due to agonist effects on the estrogen receptor, while in the uterus and mammary glands, they act as estrogen receptor antagonists. SERMs are used for the prevention and in the treatment of diseases such as osteoporosis, uterus and breast cancers (Maximov et al., 2013).

Small molecule peptides to block PPIs

Several approaches have been made to target PPIs as shown in Fig. 2. Developing small molecule inhibitors fails several times for lack of specificity, sometimes these molecules have off target effect leading to side effects while used as therapeutic drugs (Hoelder et al., 2012). Using peptides to block PPIs seems to be a very useful and efficient way of blocking PPIs with high specificity. When a protein is interacting with another protein, these interactions occur through specific domains involving fewer amino acids, and it has been demonstrated that peptides that have similar amino acid sequences as that of the interacting domain (of either protein) can inhibit a PPI by competing for the binding sites. And these peptide mediated interference in PPIs are so specific that in the whole cell where several PPIs occur, only one particular interaction can be targeted by using competitive peptides. Cell penetrating peptides such as viral TAT peptide derived from viral protein makes the task of delivering hydrophilic peptides into the cells very easily (Copolovici et al., 2014). Several modifications are made in the peptides in order to increase the half life of the peptide in the cell as well as in the plasma (Fosgerau and Hoffmann,

Table 1 List of different inhibitors inhibiting various PPIs and its applications

	Inhibitor	Interacting proteins	Effect of the inhibitor	Reference
Ligand-receptor mediated inhibition using small molecules	Lovastatin, BIRT377	LFA-1/ICAM interaction	Inflammatory diseases and graft rejection after transplantation	Kallen et al., 1999
	SB 247464	Granulocyte-colony stimulating factor stimulant	Neutropenia	Tian et al., 1998
	Ro26-4550	Interaction of IL-2 to IL-2Rα	Immuno modulation	Tilley et al., 1997
	SP4206	Interaction of IL-2 to IL-2Rα	Immuno modulation	Thanos et al., 2003
	U0126	MEK1/2 interaction with ERK	Antagonize AP1 transactivation	Favata et al., 1998
Small molecules inhibitors of PPI involved in signaling cascade	CEP-1347	Kinase activity of MLKs	Inhibits JNK signalling	Saporito et al., 2001
	Gleevec	ATP binding site of β amyloid peptide	Amyloid plaque formation in Alzheimer's	Netzer et al., 2003
	SM164-Smac mimetic	XIAP-Caspase interaction	Induction of apoptosis in cancer cells	Lu et al., 2008
	CCG-17444	Shroom3-rho kinase interaction	Axon outgrowth- neural repair	Dickson et al., 2015
	M2I-I(MAD 2 Inhibitor 1)	MAD 2/CDC20 interaction	Mitotic spindle assembly	Kastl et al., 2015
	Small molecule inhibitor	E3 ligase VHL and HIF1α interaction	Chronic anemia	Buckley et al., 2012
	PPA250	Inhibit iNOS dimerization	Tissue damage during inflammation	Ohtsuka et al., 2002
	HA 14-1	BAK BH3/BCL2 interaction	Tumor cells apoptosis	Wang et al., 2000
Ppi inhibitors preventing nuclear translocation	SC236	Rel/p65 translocation	Inflammation inducing gastric cancer	Wong et al., 2003
PPI inhibitors in the nucleus	BILD 1263	HSV-Ribonucleotide inhibitor	Prevents replication of HSV-1 and HSV-2 preventing herpes virus infections	Liuzzi et al., 1994
	Bazidoxifene	Selective estrogen receptor modulator	Treatment of post menopausal problems	Li et al., 2014

2015).A list of peptide-based inhibitors of PPIs are listed in Table 2.

Conclusion

Multicellular organisms produce various proteins, each function in the organism is mediated by specific physical interactions among those group of proteins involved. The success in developing novel therapeutic drugs lies in the identification of crucial PPIs that are involved in mediating particular function of a cell. Using various techniques to identify PPIs as mentioned above more and more PPIs need to be identified that are specific for a specific function in the cells. Later by identifying the domains involved in these secret handshakes, drugs can be developed that can interfere in a particular PPI, there by a signal transduction pathway can be manipulated and this type of drugs will serve as a novel

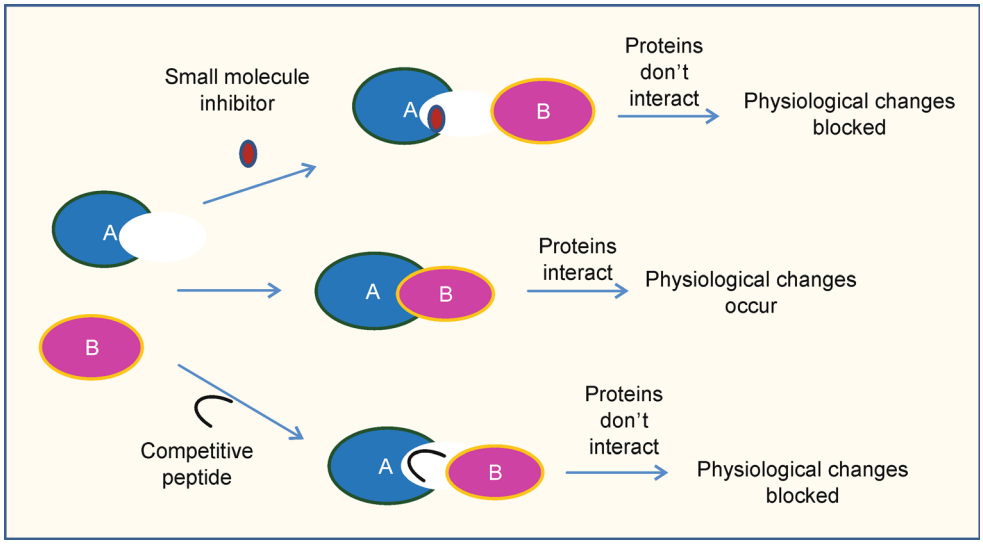

Figure 2 Different methods to target protein-protein interactions.

Table 2 Peptide inhibitors targeting PPIs at various levels and its applications

Class	Peptide	Peptide Sequence	Inhibition
Peptides inhibiting Receptor-protein interactions	Pep2-8	TVFTSWEEYLDWV	PCSK9 protein binding to LDL Receptor (Zhang et al., 2014)
	Akt in (AKT inhibitor)	AVTDHPDRLWAWEKF	TCL1-Akt interaction AKT kinase activity (Hiromura et al.,2004)
Peptides inhibiting protein interactions involved during signalling cascade	I-JIP (Inhibitor of JNK based on JIP-1)	GPGTGSGDTYRPKRPTTLNLF	JNK activity towards c-Jun, Elk and ATF-2 (Barr et al., 2002)
	NBD pepteide (NEMO-binding domain peptide)	FTALDWSWLQTE	IKKβ- NEMO interaction , inhibiting NF-κB activity (May et al., 2000)
	MEK1 peptide inhibitor	GYGRKKRRQRRRGMPKKKPTPIQLNP	ERK activation by MEK (Kelemen et al., 2001)
	TLR2-BBP	RQIKIWFQNRRMKWKKLHKRDFVPGKWIID	LPS induced ERK activation-TLR signal-ling(Toshchakov et al., 2007)
	MK2i(MK2 inhibitor)	WLRRIKAWLRRIKALNRQLGVAA	TGF-β1-induced HSP27 phosphorylation (Lopes et al., 2009)
	TAT-MKK3b peptide	YGRKKRRQRRRGKGKSKRKKDLRI	Prevents p38 activation by LPS induced TNFα secretion (Fu et al., 2008)
Peptides inhibiting nuclear translocation of proteins	TLR4-BBP	RQIKIWFQNRRMKWKKLHYRDFIPGVAIAA	LPS induced NFκB translocation and IL-1β mRNA expression (Toshchakov et al., 2007)
	PT5 peptide	QGQVVSNGKSTDEQS	TAB/p38 α interaction decreasing myo-cardial I/R injury (Wang et al., 2013)
Peptides inhibiting protein interactions in the nucleus	^{D}PMI-α	TNWYANLEKLLR	p53-MDM2 interaction (Liu et al., 2010)

therapeutic drugs with less side effect because they interfere in the secret communications of the cell.

Compliance with ethics guidelines

This manuscript is a review article and does not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

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