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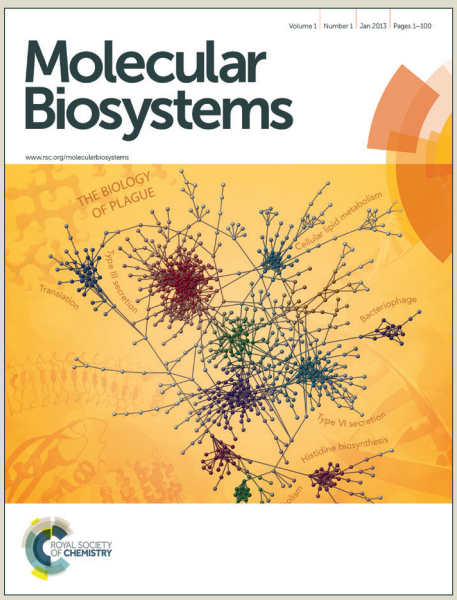

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ARTICLE

PI3K/AKT/mTOR Interactive Pathway

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The phosphatidylinositol 3-kinase (PI3K) / AKT / mammalian target of rapamycin (mTOR) signalling pathway is hyperactivated or altered in many cancer types and regulates a broad range of cellular processes including survival, proliferation, growth, metabolism, angiogenesis and metastasis. The PI3K/AKT/mTOR pathway is regulated by a wide-range of upstream signalling proteins and it regulates many downstream effectors by collaborating with various compensatory signalling pathways, primarily with RAF/MEK/ERK. Limited clinical success of the available targeted therapeutic agents and challenges mediated by tumour heterogeneity across different cancer types emphasize the importance of alterations in PI3K/AKT/mTOR pathway in the design of effective personalized treatment strategies. Here we report a comprehensive PI3K/AKT/mTOR network that represents the intricate crosstalk between compensatory pathways, which can be utilized to study AKT signalling mechanism in detail and improve the personalized combinatorial therapeutic strategies.

The phosphatidylinositol-3-kinase(PI3K) / AKT / mammalian target of rapamycin (mTOR) signalling pathway is hyperactivated in various cancer types and regulates a broad spectrum of cellular mechanisms including survival, proliferation, growth, metabolism, angiogenesis and metastasis [1–3]. The PI3K/AKT/mTOR pathway transmits signals from a wide-range of upstream regulatory proteins such as PTEN, PI3K and RTKs, and to many downstream effectors such as GSK-3β, FOXO, and MDM2, which are under control of various compensatory signalling pathways as well. The stringent control of upstream regulators and downstream effectors by feedback mechanisms further complicates the signalling pathway. In this work, we curated and compiled available interactions data from 498 peer reviewed literature and constructed a comprehensive PI3K/AKT/mTOR signalling pathway with 25 drug categories comprising 104 molecularly targeted therapeutic agents. This literature-curated interaction data summarizes the PI3K/AKT/mTOR signalling pathway and illustrates the feedback loops and the intertwined interactions with other signalling pathways.

The compiled PI3K/AKT/mTOR signalling pathway consists of 254 components and 478 links between them. A complete view of the constructed signalling pathway is illustrated in Figure 1 where each signalling component is represented with a node and each line between them is represented with an edge (for interactive visualization and edit, see the Cytoscape session file in the Supplementary Information 1). In Figure 1, Node shapes and colours represent the types and cellular localizations of proteins, respectively (for details of the visualization, see Table 1). The size of the protein node represents the centrality and the connectivity in the pathway. Nodes are cross-referenced to NCBI Gene, Swiss-Prot, and PDB identifiers in addition to their gene symbol and protein names. Attributes of interaction edges are complemented with the literature reports. Stimulatory and inhibitory edges ultimately regulate 17 cellular processes: Actin cytoskeleton, Angiogenesis, Apoptosis, Autophagy, Blood cell development, Cell cycle progression, Cell survival, DNA repair, Epigenetic regulation, Genetic Stability, GTP biosynthesis, Ion transport, Metabolism, Metastasis, Protein synthesis, Regulation of gene expression, and Ribosomal RNA synthesis. Detailed view of some of the critical hubs, which are proteins having many connections in the network, and processes in this pathway are provided in Figure 2.

Class IA PI3Ks are heterodimers of a catalytic subunit (p110) and a regulatory subunit (p85). PI3Ks are stimulated by activated tyrosine kinase receptors (RTK), G protein-coupled receptors (GPCR) or by constitutively activated RAS [2, 4–9]. Ras oncogene is a monomeric membrane-associated GTP-binding protein that binds and activates several effector proteins including PI3K and RAF. The RAF kinase transduces signal through a mitogen-activated protein kinase (MAPK) cascade (MEK/ERK). The PI3K/AKT/mTOR and RAF/MEK/ERK signalling cascades are compensatory pathways that mediate cell survival through co-regulated proteins. Signalling through these pathways initiates primarily at the cell surface receptors, flow through cytoplasmic kinases, phosphatases and various molecular switch proteins, which ultimately leads to controlled gene expression within the nucleus. In the nucleus, transcription factors promote expression of genes that facilitate tumour growth and further stimulate negative feedback mechanisms to suppress the expression of growth factor receptors. Throughout this signal transduction, PI3K/AKT/mTOR and RAF/MEK/ERK pathways negatively regulate each other. AKT directly phosphorylates and inactivates RAF, while MEK suppresses PI3K signalling by promoting membrane localization of phosphatase and tensin homologue (PTEN) [3, 10–13].

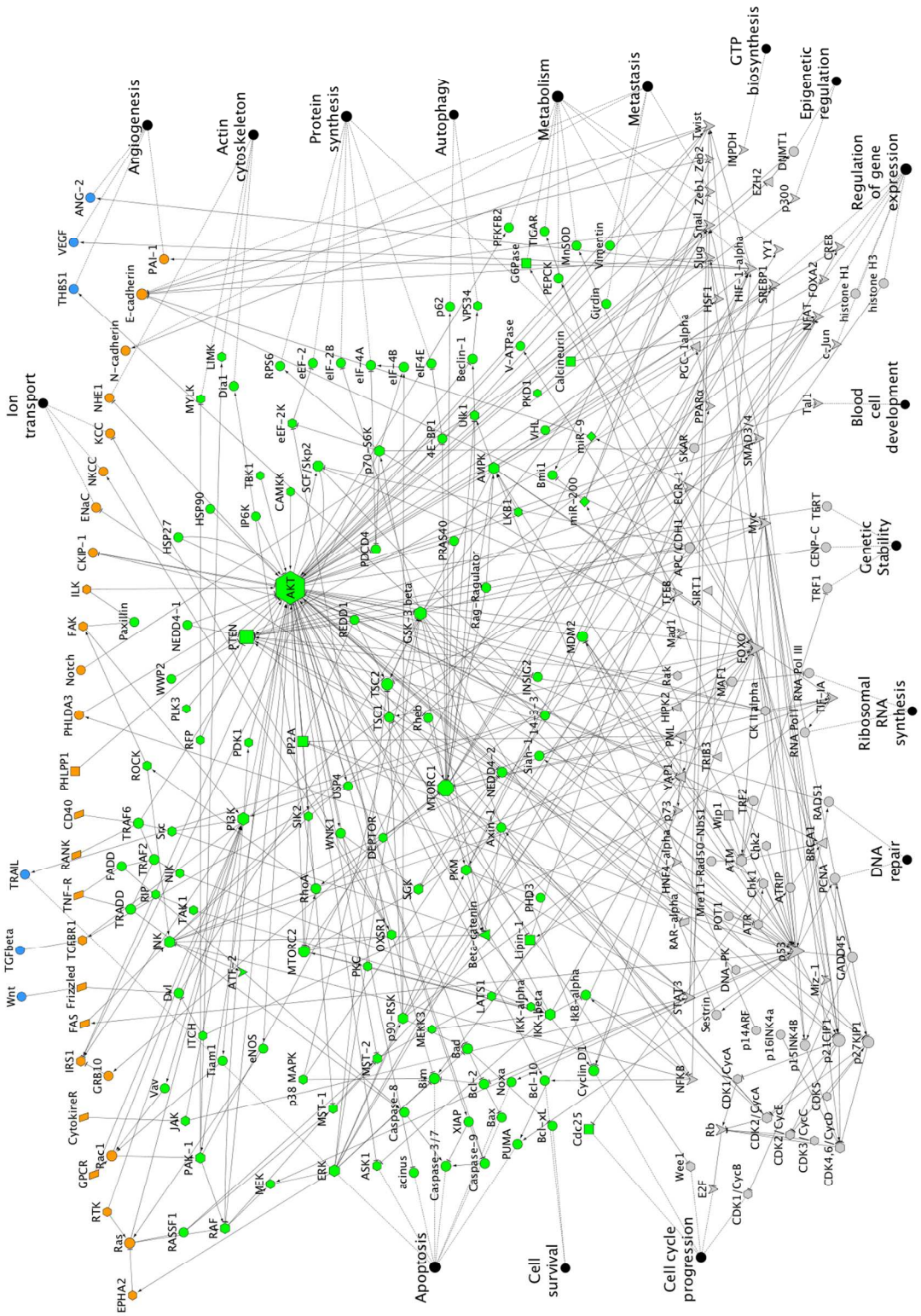
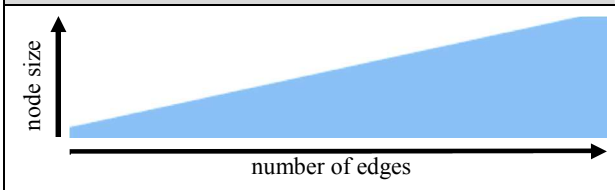


Fig. 1 PI3K/AKT/mTOR Pathway. Activation, inhibition, interaction and regulation edge categories represent the relationships between the protein nodes (Cytoscape file, Supplementary Information 1). Detailed legend is given in Table 1.

Class IA PI3Ks convert phosphatidylinositol□4,5□ bisphosphate (PIP2) lipids to phosphatidylinositol□3,4,5□ trisphosphate (PIP3) at the membrane. PIP3 provides docking sites for 3-phosphoinositide-dependent kinases, PDK1 and mTORC2 (PDK2), which in turn phosphorylate the AKT serine/threonine kinase at Thr-308 and Ser-473, respectively, thereby leading to its activation [14–17]. Activation of AKT by PI3K is antagonized by the cytoplasmic PTEN, which dephosphorylates PIP3 [18]. Once translocated into the nucleus, the tumour suppressor PTEN regulates chromosomal stability and DNA repair response [19]. AKT protein is also regulated negatively by PP2A, PHLPP1, PHLDA3, CKIP-1, IP6K, NEDD4-1 and TRIB3 and positively by DNA-PK, CAMKK, HSP27, HSP90, ILK, TBK1 and CDK2 [20–35]. Hyperactivation of RTKs through mutations or over-expression upregulate also the activity of PI3K/AKT/mTOR pathway. The most common alterations in the PI3K/AKT/mTOR pathway, which lead to constitutive signalling, are the loss of the tumour suppressor PTEN by mutation or deletions, activating mutations of the PIK3CA gene (p110α), activating mutations of the PIK3R1 gene (p85α) and the somatic mutations in AKT1, AKT2 and AKT3 genes [2, 18, 36, 37].

Table 1 Legend of the PI3K/AKT/mTOR Pathway

Node Type	Node #	Node Shape
Protein	107	
Kinase	65	
Phosphatase	8	
Signalling Receptor	8	
Transcription Factor	33	
Transcription Regulator	9	
micro-RNA	2	
Complex	5	
Cellular process	17	
Drug categories	25	
Node Localization	**Node #**	**Node Color**
Extracellular	6	blue
Plasma membrane	27	orange
Cytoplasm	123	green
Nucleus	81	gray
Node Size		
		
Edge Type	**Edge #**	**Edge Line**
activation	222	
inhibition	169	
interaction	22	
regulation	65	

Once activated, AKT mediates cell growth and survival by phosphorylating various cytoplasmic proteins. The major downstream effector is the serine/threonine kinase mTOR, which also senses nutrient levels, presence of growth factors, and cellular energy status and mediates several catabolic and anabolic processes to maintain metabolism and cell growth [38]. mTOR forms two multi-protein complexes, Raptor associated complex mTORC1 and Rictor associated complex mTORC2. AKT inactivates TSC1-TSC2, and thereby promotes Rheb GTPase to activate mTORC1 [39]. Subsequently, active mTORC1 promotes i) protein synthesis by inactivating the translational inhibitor 4E-BP1 and by activating the kinase S6K, ii) induces lipid biogenesis by activating SREBP1 and PPARγ transcription factors and iii) inhibits autophagy by blocking ULK1 [38, 40]. On the other hand, mTORC2 directly activates AKT by phosphorylating at Ser-473 [15]. Other major downstream effectors inhibited by AKT are the metabolic regulator glycogen synthase kinase-3-β (GSK-3β), the pro-apoptotic proteins Bad, Bim and caspase 9, the cell cycle regulators p21 and p27, and the forkhead box O (FOXO) family transcription factors [41–57]. Phosphorylation and inactivation of FOXO transcription factors suppresses the expression of growth factor receptors which acts as a negative feedback AKT kinase pathway [52–56]. The major downstream effectors activated by AKT are the NF-κB regulator IKK-α, the p53 inhibitor MDM2 and the genetic stability guardian telomerase reverse transcriptase (TERT) and cAMP responsive element binding protein 1 (CREB) transcription factor [58–65]. Many downstream targets of AKT are also co-regulated by ERK. Redundant functions of ERK and AKT kinases include activation of CREB, and inhibition of GSK-3β, Bim, Bad and TSC2. Hence, ERK and AKT signalling can compensate each other in the activation of cell survival processes. Both pathways regulate pro-survival gene expression through Bmi1 polycomb ring finger and c-Jun oncogenes, H1 and H3 histones and transcription factors CREB, nuclear factor of activated T-cells (NFAT) and FOXO [66, 67]. Pro-survival gene expression is also under control of epigenetic regulation through the DNA methyltransferase DNMT1 and the histone acetyltransferase p300 [68, 69].

The differentiation of epithelial cells into motile mesenchymal cells, known as epithelial–mesenchymal transition (EMT), promotes metastasis. The key transcription factors that mediate AKT-stimulated metastasis are Twist, Snail, Slug, Zeb1, and Zeb2 [70, 71]. The AKT kinase also regulates Vimentin, Girdin, N-cadherin and E-cadherin to enable metastasis. Cells that undergo EMT reorganize their actin cytoskeleton to facilitate cell motility [72]. Activated mTORC1 initiates pro-metastatic actin cytoskeleton remodeling by activating RhoA and its associated kinase ROCK. Consequent activation of Diaphanous-Related Formin 1 (Dia1), LIM kinase (LIMK) and myosin light chain kinase (MYLK) drives actin reorganization [73, 74]. These cytoskeletal changes are also associated with PI3K/AKT/mTOR signalling mediated alterations in focal adhesion kinase (FAK), Paxillin and sodium/hydrogen exchanger member 1 (NHE1) proteins.

Rapidly growing and spreading tumour cells require oxygen and nutrients, which are supplied by angiogenesis [75]. During angiogenesis and neo-vascularization, interactions between tumour cells and vascular endothelial cells are mediated by the PI3K/AKT/mTOR signalling through Hypoxia induced factor 1 alpha (HIF-1α) and the angiogenic factors ANG-2 (angiopoietin 2), plasminogen activator inhibitor type 1 (PAI-1), Thrombospondin1 (THBS1) and vascular endothelial growth factor (VEGF) [76]. Moreover, cancer cells re-program their metabolism to maintain high rates of aerobic glycolysis for ATP generation, increase macromolecule biosynthesis and maintain redox homeostasis [77, 78]. The PI3K/AKT/mTOR network regulates metabolism through glucose-6-phosphatase

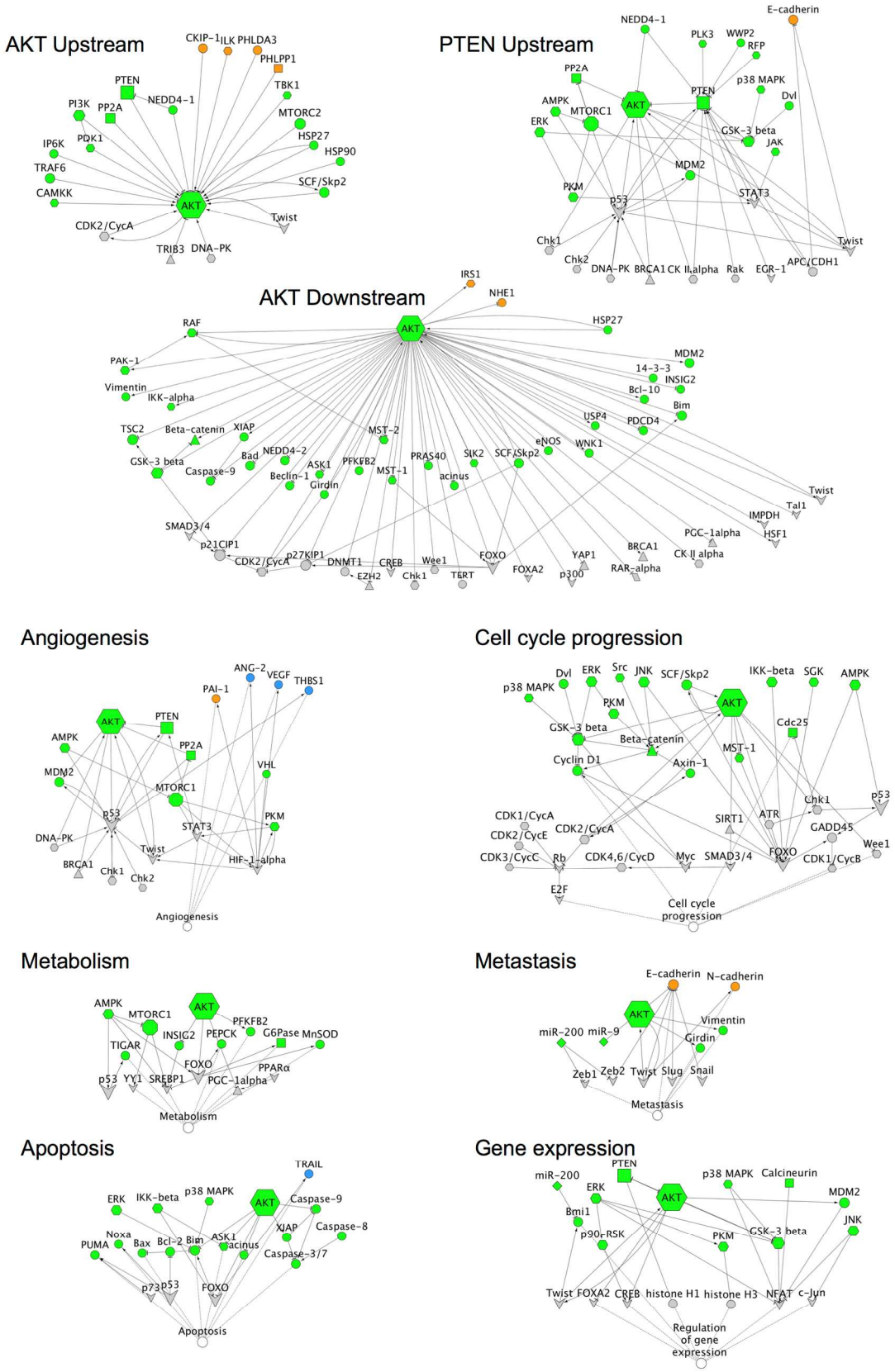


Fig. 2 Interaction partners of hub proteins and processes.

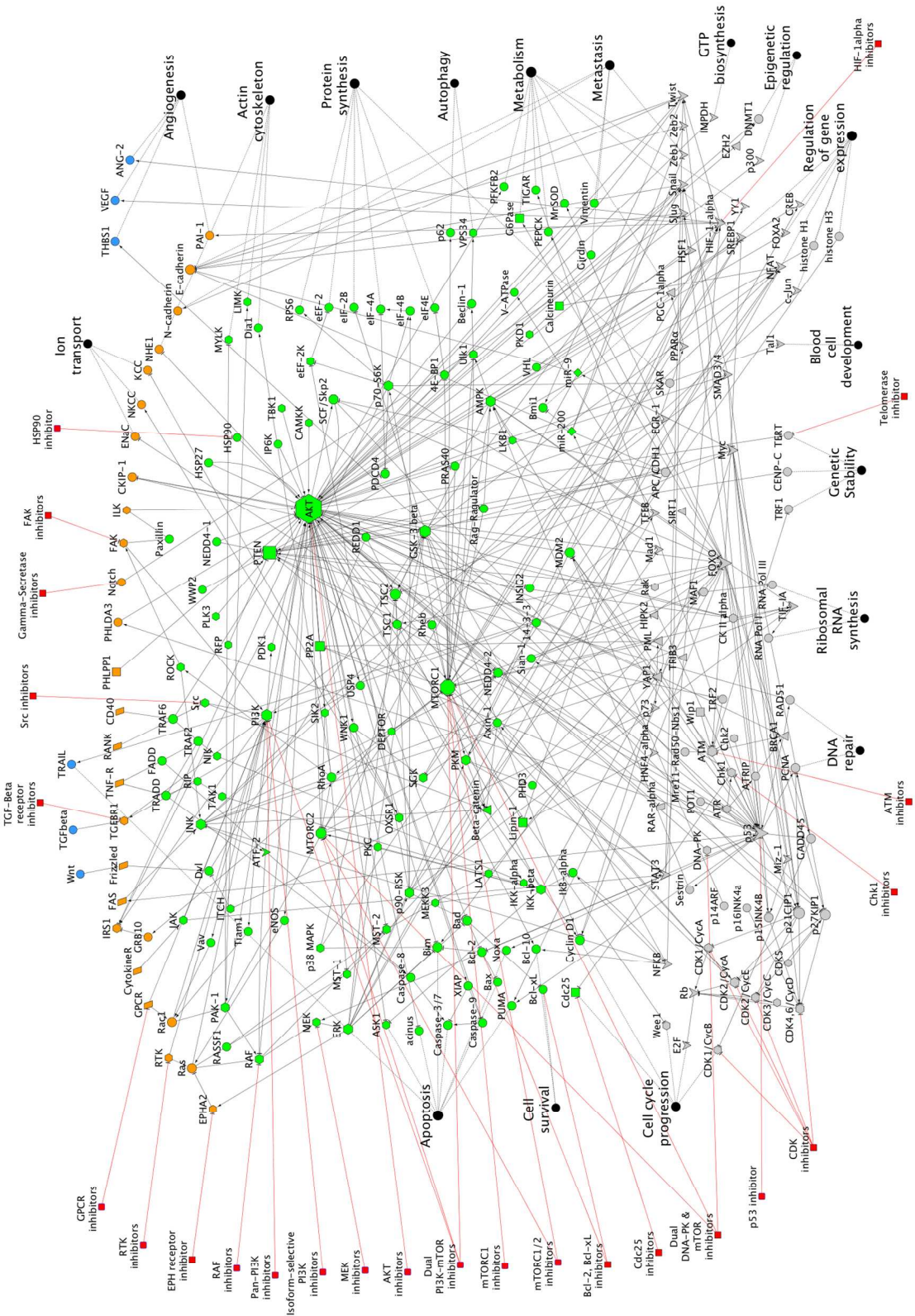


Fig. 3 PI3K/AKT/mTOR with 25 drug categories. Supplementary Cytoscape file maps 104 therapeutic agents on their protein targets (Supplementary information 2).

(G6Pase), superoxide dismutase 2 (MnSOD), phosphoenolpyruvate carboxykinase 2 (PEPCK), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 2 (PFKFB2), peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α), sterol regulatory element binding transcription factor 1 (SREBP1), TP53-Induced Glycolysis And Apoptosis Regulator (TIGAR) and YY1 transcription factor. In addition, PI3K/AKT/mTOR signalling regulates autophagy through Beclin1, sequestosome 1 (p62) and phosphoinositide-3-kinase, class 3 (VPS34) in order to enable fast growing cells to break down cellular organelles, resulting in recycled catabolites that can be used for biosynthesis and energy metabolism [78].

p53 has been known as the guardian of the genome for decades [79]. Recent findings identified PTEN as a direct transcriptional target of p53 [80]. In turn, PTEN physically associates with p53 and regulates its transcriptional activity [81–83]. Hence, constitutive activation of PI3K/AKT signalling by loss of PTEN or activating mutations in PIK3CA can stimulate p53 activity [84]. Furthermore, active AKT signalling maintains genetic stability through TERT and telomeric repeat binding factor (TRF1) [64, 65].

Loss of the PTEN and TP53 genes is frequently observed in various types of cancer and cancers that harbour both alterations exhibit a more aggressive phenotype [85–87]. Since PTEN regulates its own expression by stabilizing p53 and regulates DNA damage repair and genomic stability, it is proposed as a new guardian of genome integrity [88]. PTEN physically associates with CENP-C at centromeres and loss of PTEN results in centromere breakage and chromosomal translocations [89]. Moreover, PTEN deficiency leads to down-regulation of RAD51 and thereby creates a homologous recombination (HR) defect, which results in the accumulation of DNA double-strand breaks (DSBs). DSB repair is mediated majorly by the BRCA1 gene, which is activated by AKT and the DNA damage response regulator kinase Ataxia-telangiectasia mutated (ATM) [90–93]. The HR repair deficiency, which is caused by the loss of either PTEN or BRCA1 genes, sensitizes cancer cells to DNA damage-inducing therapeutic agents and to the inhibitors of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP) [94, 95]. However, such therapeutic strategies are not applicable to cancers with both alterations [96]. PTEN is frequently mutated in BRCA1-deficient tumours, leading to constitutive signalling from AKT [97]. Simultaneous loss of PTEN and BRCA1 confers further resistance to PARP inhibitors [95]. Yet, simultaneous loss of PTEN and p53 are sensitive to combinational treatment with PARP and PI3K inhibitors [85]. Therefore, it is essential to identify the signalling profile of each individual tumour before choosing the best therapeutic approach.

The comprehensive PI3K/AKT/mTOR pathway described in this report covers 25 drug categories, which include 104 molecularly targeted therapeutic agents undergoing clinical development, that were mapped on their protein targets (Figure 2). Despite the evident cytotoxic effect of PI3K/AKT inhibitors in cancer cells having either basal level or hyperactive AKT signalling, the therapeutic efficacy of these inhibitors are limited due to off-target toxicities and initiation of negative feedback loops that cause re-activation of upstream RTK signalling [98, 99]. Indeed, rapid induction of apoptosis by PI3K inhibitors can be explained by their ability to cause a transient inhibition of RAS/RAF/MEK/ERK signalling [100]. Compensatory ERK pathway activation in response to AKT pathway inhibition is one of the reasons for therapeutic resistance [101–103]. RAF kinase inhibitors are the most studied anti-cancer drugs with substantial therapeutic outcomes. However, their therapeutic effects are often temporary, since cancer cells acquire resistance to RAF inhibitors and promote tumour recurrence [104]. Therapeutic limitations of single agents in the clinic arise from such rapid but transient cytotoxic responses due to the adaptive capabilities of signalling networks to enable sustained signalling from untargeted compensatory pathways [105–108]. Resistance to molecularly targeted therapeutics is a major obstacle in the design of effective treatment strategies due to the complex crosstalk and feedback mechanisms within and between signal transduction pathways [109]. Therefore, combining signal transduction inhibitors for simultaneous targeting of compensatory pathways with redundant functions will be the most effective strategy to overcome resistance and prevent tumour recurrence. The dual-targeting strategy involving PI3K/AKT/mTOR and RAF/MEK/ERK pathways in patients with advanced cancer has shown encouraging therapeutic outcome [110]. In order to design effective combination therapies for durable clinical outcomes, the intricate crosstalk between compensatory pathways should be studied through comprehensive network representations and analysis.

PI3K/AKT/mTOR and RAS/RAF/MEK pathways are the major oncogenic pathways in human tumors, harboring

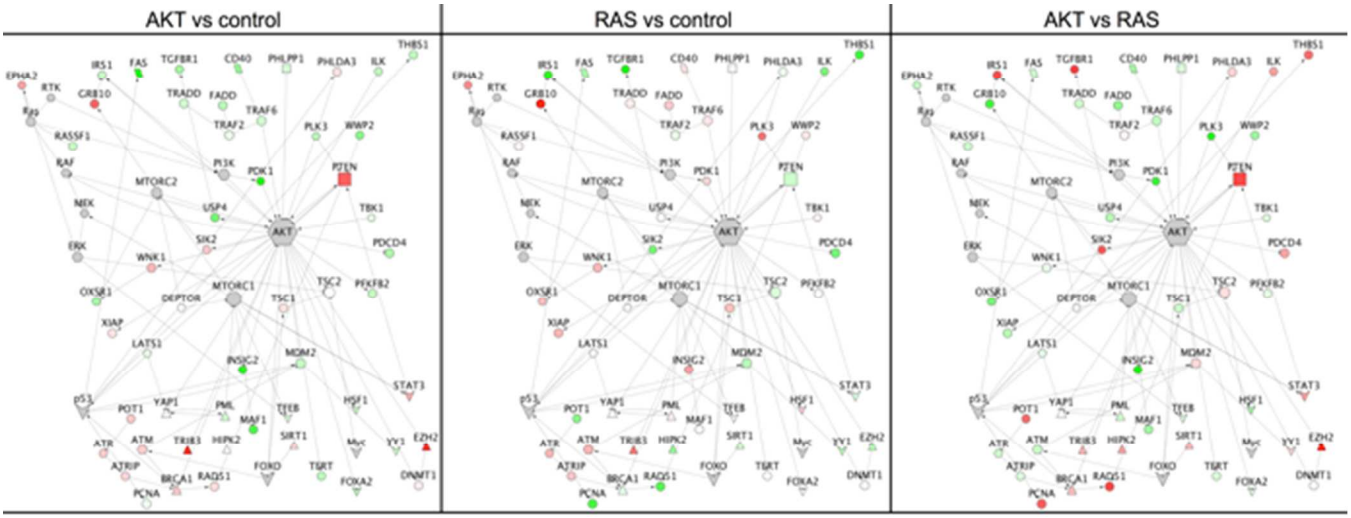


Fig. 4 Comparative visualization of the gene expression profiles of fibroblasts with oncogenic AKT and RAS (GSE45276). Up-regulated genes are colored in red, down-regulated genes are colored in green.

frequent alterations in the PIK3CA and RAS oncogenes and the PTEN tumor suppressor protein. Yet, even in the absence of oncogenic alterations, several feedback mechanisms can enhance signaling from these networks. The main strategy to overcome the feedback signaling is the simultaneous inhibition of multiple signaling nodes within and between pathways. This literature compiled and curated PI3K/AKT/mTOR network, which also incorporates crosstalk between PI3K signaling and other oncogenic pathways, will enable visualization and comparative analysis of omics data. The mapping of high-throughput transcriptomics or proteomics data to this pathway will assist identification of new drug targets. Moreover, in-depth analysis of all components of the pathway can reveal the state of oncogene addiction in individual cancers and thereby facilitate personalized medicine to target the "Achilles' heel" in specific cancers [111–113]. For instance, gene expression profiling of fibroblasts with oncogenic AKT and RAS show that the common downstream signaling components are altered differentially depending on the oncogene [114]. Activation of AKT leads to up-regulation of the PTEN tumor suppressor as a negative feedback mechanism (Figure 4). Oncogenic AKT also leads to down-regulation of membrane receptors and up-regulation of DNA repair genes. On the contrary, oncogenic RAS down-regulates PTEN and some of the DNA repair genes. The major hub components of the signaling pathway such as MTORC1, MTORC2, RAF, MEK, ERK, P53 and FOXO are not altered. This further emphasized the importance of visualizing the less-studied intermediate components of the signaling pathway.

Pathway maps can be further enhanced by integrating structural aspects of proteins. Although the classical graph theoretical node/edge representation improves our knowledge about the signal transduction, high resolution three-dimensional structural data is crucial to learn about *how* proteins interact. Mapping structural data onto classical pathways, in other words, constructing structural pathways is necessary for a rational pathway analysis. A structural pathway can be utilized for discovering and optimizing therapeutic agents, predicting their side-effects, identifying protein targets in disease, finding binding preferences of proteins, understanding the exertion of the function and revealing genotype-phenotype relations. The number of protein complexes deposited in Protein Databank (PDB) is increasing exponentially [115]. However, this data is sparse and prediction approaches are necessary to model structurally unknown interactions. There are several well-established approaches to predict protein complexes [116–120]. Among them, the method in Interactome3D uses global and domain similarities of target protein pairs to the known protein complexes [117]. Another method, PRISM, uses evolutionary and structural similarity of target protein pairs with known protein interfaces and refines the predicted complexes by optimization and energy calculation [118]. These methods have been extensively used to construct structural pathways and shown how the structural data can enhance our understanding in pathway analysis. *Tuncbag et al* have constructed structural p53 pathway and illustrated the multi-interface hub character of p53 and Mdm2 proteins using the data retrieved from PDB and PRISM [121]. *Mosca et al* have revisited the complement cascade pathway in KEGG by integrating the both experimental and predicted structural information of protein interactions deposited in Interactome3D. Using the structural pathway, they could find the location of disease mutations in proteins and comment out if the mutation has any effect on the binding. Also, order of events in the complement cascade pathway has been illustrated by identifying simultaneously possible and mutually exclusive interactions [117]. In another study, human ubiquitination pathway has been modelled at the proteome scale and it has been shown that targeting E3s in this pathway could be a good approach in many diseases [122]. Also, structural pathways in breast cancer have been constructed and known mutations have been mapped onto protein interfaces in these pathways to elucidate the metastasis mechanism to brain and lung [123]. Structural data integration has been applied to the Interleukin-1 initiated signaling pathway to show the mechanistic details of interactions and effect of SNPs on these interactions [124]. The structural network of ERKs in the MAPK pathway has been constructed and time dependency in this pathway model has been illustrated by identifying simultaneously possible interactions and competing interactions by referring to the binding regions and spatial constraints [125].

PI3K/AKT/mTOR pathway we described here can be further enhanced by integrating structural data on proteins and constructing a structural network. The structural PI3K/AKT/mTOR pathway can rationalize the mechanistic details of interactions, mutations, order of events within the pathway and genotype-phenotype relations. We believe that PI3K/AKT/mTOR pathway we described in this work will be of interest to the community for the multiscale studies.

Conclusions

Literature curated pathway data are invaluable for systems biology approaches to analyze and understand the molecular mechanisms of human cancers. Although PI3K/AKT/mTOR pathway is described as one of the pathways, which is highly altered in various cancers, there is not a comprehensive pathway data entry devoted to systems biology analyses. The literature compiled, curated and carefully crafted interactions presents a detailed map of PI3K/AKT/mTOR pathway with drug categories that can be exploited for understanding the role of this pathway in various cellular processes. Association of each interaction with their PubMed IDs and annotation of each gene/protein node with their Entrez, SwissProt, PDB IDs and EC numbers provides resources for in-depth analyses of this pathway. Furthermore interactive visualization of the pathway can be used for *in silico* gene deletion/perturbation experiments to simulate the response to potential targeted therapeutics, to foresee off-target effects, hence facilitate the development of novel therapeutic agents.

Notes and references

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† Electronic Supplementary Information (ESI) available: [Supplementary information 1: Cytoscape session file of the pathway in Figure 1, Supplementary information 2: Cytoscape session file of the pathway including therapeutic agents in Figure 2, Supplementary information 3: References of interactions from literature curation]. See DOI: 10.1039/b000000x/

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