

# Identification of Top-ranked Proteins within a Directional Protein Interaction Network using the PageRank Algorithm: Applications in Humans and Plants

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## Abstract

Somatic mutation of signal transduction genes or key nodes of the cellular protein network can cause severe diseases in humans but can sometimes genetically improve plants, likely because growth is determinate in animals but indeterminate in plants. This article reviews protein networks; human protein ranking; the mitogen-activated protein kinase (MAPK) and insulin (phosphoinositide 3-kinase [PI3K]/phosphatase and tensin homolog [PTEN]/protein kinase B [AKT]) signaling pathways; human diseases caused by somatic mutations to the PI3K/PTEN/AKT pathway; use of the MAPK pathway in plant molecular breeding; and protein domain evolution. Casitas B-lineage lymphoma (CBL), PTEN, MAPK1 and PIK3CA are among the top-ranked proteins in directional rankings. Eight proteins (ACVR1, CDC42, RAC1, RAF1, RHOA, TGFBR1, TRAF2, and TRAF6) are ranked in the top 50 key players in both signal emission and signal reception and in interaction with many other proteins. Top-ranked proteins likely have major impacts on the network function. Such proteins are targets for drug discovery, because their mutations are implicated in various cancers and overgrowth syndromes. Appropriately managing food intake may help reduce the growth of tumors or malformation of tissues. The role of the protein kinase C/fatty acid synthase pathway in fat deposition in PTEN/PI3K patients should be investigated. Both the MAPK and insulin signaling pathways exist in plants, and MAPK pathway engineering can improve plant tolerance to biotic and abiotic stresses such as salinity.

## Abbreviations

2DE	two-dimensional gel electrophoresis
3'UTR	three-prime untranslated region
αPKC	alpha protein kinase C
ACVR1	activin A receptor, type I
ADAM19	A disintegrin and metalloproteinase 19
AGGF1	angiogenic factor with G patch and FHA domains 1

AKT	protein kinase B, serine/threonine kinase (Akt/PKB)
CBL	Casitas B-lineage lymphoma, an E3 ubiquitin-protein ligase
CDC42	cell division cycle guanosine-5'-triphosphate-binding protein 42
CLOVES	congenital lipomatous overgrowth, vascular malformations, epidermal nevis, spinal/skeletal anomalies/scoliosis (syndrome)
ERK	extracellular-signal-regulated kinase
FAS	fatty acid synthase
FDH	formate dehydrogenase
GAP	GTPase-activating protein
GDP	guanosine-5'-diphosphate
GnRH	gonadotropin-releasing hormone
GTP	guanosine-5'-triphosphate
HHML	hemihyperplasia-multiple lipomatosis (syndrome)
HTLVI	human T-lymphotropic virus type I
IGFI	insulin-like growth factor I
INS	insulin
IRS	insulin receptor substrate
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MAPK	mitogen-activated protein kinase
MAPKK	mitogen-activated protein kinase kinase
MAPKKK	mitogen-activated protein kinase kinase kinase
MAPKKKK	mitogen-activated protein kinase kinase kinase kinase
miRNA	microRNA
mRNA	messenger RNA
mTOR	mechanistic target of rapamycin (serine/threonine kinase) (also known as mammalian target of rapamycin)
NADH	reduced nicotinamide adenine dinucleotide
NOD	nucleotide-binding oligomerization domain
PageRank	algorithm used by Google Search (named after Larry Page)
PDK	phosphoinositide-dependent kinase
PI3K	phosphoinositide 3-kinase (synonym: phosphatidylinositol 3-kinase)
PiII or Pi-II	type II proteinase inhibitor
PIK3CA	
or PIK3Ca	p110alpha catalytic subunit of phospho-inositide 3-kinase
PIP2	phosphatidylinositol (3,4)bisphosphate
PIP3	phosphatidylinositol (3,4,5)trisphosphate
PKB	protein kinase B
PKC	protein kinase C
PROS	PIK3CA-related overgrowth spectrum (syndrome)
PTEN	phosphatase and tensin homolog
RAC1	ras-related, small guanosine-5'-triphosphate-binding protein (rho family)
RAF1	vraf1 murine leukemia viral oncogene homolog 1
RHOA	ras homolog gene family, member A
RIGI	retinoic acid-inducible gene I
SIMKK	stress-induced mitogen-activated protein kinase kinase
SREBP	sterol regulatory element-binding protein
TGF	transforming growth factor
TGFBR1	transforming growth factor-beta receptor 1
TNF	tumor necrosis factor
TRAF2	tumor necrosis factor receptor-associated factor 2
TRAF6	tumor necrosis factor receptor-associated factor 6, E3 ubiquitin-protein ligase
TRPV2	transient receptor potential cation channel subfamily V, member 2
TSC	tuberous sclerosis complex, including tuberous sclerosis complexes 1 (TSC1) and 2 (TSC2)
VEGF	vascular endothelial growth factor
YFP	yellow fluorescent protein
ZmS6K	maize ribosomal S6 protein kinase

### Protein domain interaction, protein networks, and protein ranking

The protein interaction network, on the proteome scale, is the protein interactome or the whole set of protein–protein interactions and chiefly determines the function and growth of the cell or the individual organism. A protein domain is a structural or functional subunit of a protein. Differing degrees of phenotypic consequences are expected for mutations on different proteins. Certain proteins have major impacts on the protein network function. Selectively targeting central nodes of the networks is an approach that has been proposed to develop drugs to kill cancer cells (Csermely et al., 2013). Various ranking approaches have been applied in analyzing protein networks (Weston et al., 2004, 2006; Singh et al., 2008; Chen et al., 2011; Iván and Grolmusz, 2011; Krallinger et al., 2011; Du et al., 2012; Cao et al., 2014). We hypothesize that top-ranked proteins have, in general, higher impacts on the network function than low-ranked proteins do. Further research should be conducted to confirm this hypothesis.

Interaction between proteins occurs usually through their functional domains (conserved functional regions) or motifs (structural characteristics) (Bhattacharyya et al., 2006). There might be over a million instances of peptide motifs in the human proteome (Tompa et al., 2014). Proteins seldom work alone to carry out their functions. Rather, proteins almost always interact with other proteins. For those involved in signal transduction, such interaction can be very transient. Given that most proteins are multidomain proteins and that an interaction between two proteins most often involves only a pair of constituent domains (one from each protein), understanding protein interactions at the domain level becomes critical to understanding the binding interfaces and the impact of binding on the effectiveness of signal transduction. Determining proteins that interact and establishing protein interaction networks (Schwikowski et al., 2000; Ito et al., 2001; Polden et al., 2011) require major experimental and bioinformatics efforts, such as the use of yeast two-hybrid screening (Vidal et al., 1996) and affinity purification and quantitative mass spectrometry (Kaake et al., 2010). A combination of high-throughput experimental technologies and computation greatly accelerates the process. Although it is challenging to trace how novel protein domains were generated from existing ones, Li et al. (2011) were able to conclude that all the intermediate domains during the generation of type II proteinase inhibitor (PIII)-7C/6C domains in the potato (*Solanum tuberosum*) proteinase inhibitor family were likely functional because the intermediate protein domains responded to selection during the emergence of that group of closely related PIII-7C/6C novel domains.

Protein interaction can be directional, particularly in signal transduction pathways. The input of a signal (e.g., recognition of a pathogen-derived protein by host cells) may trigger a protein kinase cascade (e.g., mitogen-activated protein kinase cascade), where a series of kinases are activated in a specific order (Xing et al., 2002). Phenotypic analysis of mutants was applied to decipher the direction of protein interactions in ethylene signal transduction pathways in *Arabidopsis* (Schaller and Kieber,

2002). From this perspective, protein interaction is often directional. However, most established protein networks often lack directional information, because it is often difficult to determine whether the protein–protein interaction is directional in the signal flow or whether two proteins are simply sticking together, on the basis of *in vitro* experiments using approaches such as yeast two-hybrid screening. Very often, additional experiments in a biological context are necessary. A large, proteome-wide protein network with predicted directions of protein interaction signal flow and pathways has been established using protein interaction directional scores for 2237 human proteins, including more than 300 proteins likely involved in feedback-like pathways (Liu et al., 2009). This large interactive network, which Liu et al. (2009) calls the "integrated human directional protein interaction network", contains not only most of the known signal transduction proteins but also proteins or domains (such as Fc fragment of immunoglobulin E, skeletal muscle actin ACTA1, and histone acetyltransferase MYST2) that have directional interactions that activate or are activated by other proteins or domains (Liu et al., 2009). Du et al. (2012) used the Google PageRank algorithm (Brin and Page, 1998) to analyze this protein interaction network.

This article reexamines the the top ranked proteins (Du et al. 2012) from the integrated human directional protein interaction network of Liu et al. (2009). We used the PageRank algorithm (Brin and Page, 1998) with the classical PageRank setting (forward ranking: credit given to signal receivers) and two modified credit settings (reverse ranking and non-directional ranking) (Du et al., 2012). From the original interaction network of Liu et al. (2009), we generated three credit-based protein interaction networks: a forward-ranking network (credit given to information receivers), a reverse-ranking network (credit given to information emitters), and a non-directional-ranking network (credit given to both information emitters and receivers). The forward, reverse, and non-directional rankings were achieved by ranking proteins within each of these three credit-based networks, respectively, using the PageRank algorithm. To the best of our knowledge, this was the first time that PageRank algorithms have been used for protein ranking.

We present the key nodes, the systematic differences between signal emission and signal reception, and the relative differences in degree of conservation between high- and low-ranked proteins in the human protein network. Of the top50-ranked proteins, most belonged to two critical signaling pathways: the mitogen-activated protein kinase (MAPK) pathway and the insulin (phosphatase and tensin homolog [PTEN]/protein kinase B [AKT]) pathway. These two key pathways, as well as their involvement in somatic mutation diseases in humans and their potential use in molecular breeding to enhance plant tolerance to biotic and abiotic stresses, are elaborated and discussed. Directional PageRank analysis for the human protein network was conducted based on the direction of protein activation (information flow) between protein domains. Therefore, we also briefly discuss protein domain

evolution in plants using the potato type II proteinase inhibitor (Pi-II) family as an example.

### Systematic differences in protein rankings between signal emission and signal reception

In the Google PageRank algorithm (Brin and Page, 1998), nodes tend to be ranked higher whenever the number and quality of their incoming links are higher. Du et al. (2012) called this type of ranking "PageRank forward ranking". In contrast, in what Du et al. (2012) called "PageRank reverse ranking", nodes tend to be ranked higher whenever the number and quality of their outgoing links are higher. Moreover, in non-directional ranking, nodes tend to be ranked higher whenever the total number of their incoming and outgoing links combined is higher (Du et al., 2012). When this algorithm is applied to a protein network, the top-ranked proteins in forward ranking are key "workers" or key information receivers, because those proteins are the large nodes receiving signals or information flows from other proteins or receiving actions from proteins that are also large nodes in the network. The top-ranked proteins in reverse ranking are top information emitters or regulators, because they are the large nodes that act on many other proteins. The top-ranked proteins (largest-degree nodes) in non-directional ranking are involved chiefly in the highest number of connections with other proteins.

The regulation system (information-emitting system) has some top-ranked regulators that may or may not be the largest nodes in the protein network, whereas the actual worker system (information receivers, information users) shows quite good correlation between rank position and node degree (Du et al., 2012). This situation is similar to the organization of a company, where the decision-making group (president, vice presidents, etc.) may or may not give orders directly to many people, but the workshop managers directly supervise workers to do the work. A larger task usually needs a larger number of workers. Similar to an industrial organization system, there is a systematic difference between information emitting and information receiving in the cellular protein network (Figure 1) (Du et al., 2012). The top-ranked proteins are not necessarily the largest nodes in the protein network (Du et al., 2012). For both medical drug development and plant molecular breeding, the message here is that the right protein to be targeted, in order to achieve a specific task in research, is not necessarily the largest-degree node in the protein network.

### Ranking position and degree of conservation

Top-ranked proteins in either forward ranking or reverse ranking are more conserved than mid- or low-ranked proteins, whereas the degree of conservation between human and *Caenorhabditis elegans* is not statistically different among top-, mid-, and low-ranked proteins when the ranking is non-directional (Du et al., 2012). The results suggest the following: i) high-ranked proteins, which are likely functionally important, are more conserved; ii) directional ranking, either forward or reverse, reflects the functional importance of the proteins more than non-directional ranking does; and iii) directional ranking position

is more reliable than estimation based purely on node size is.

### High-ranking proteins in the human protein network

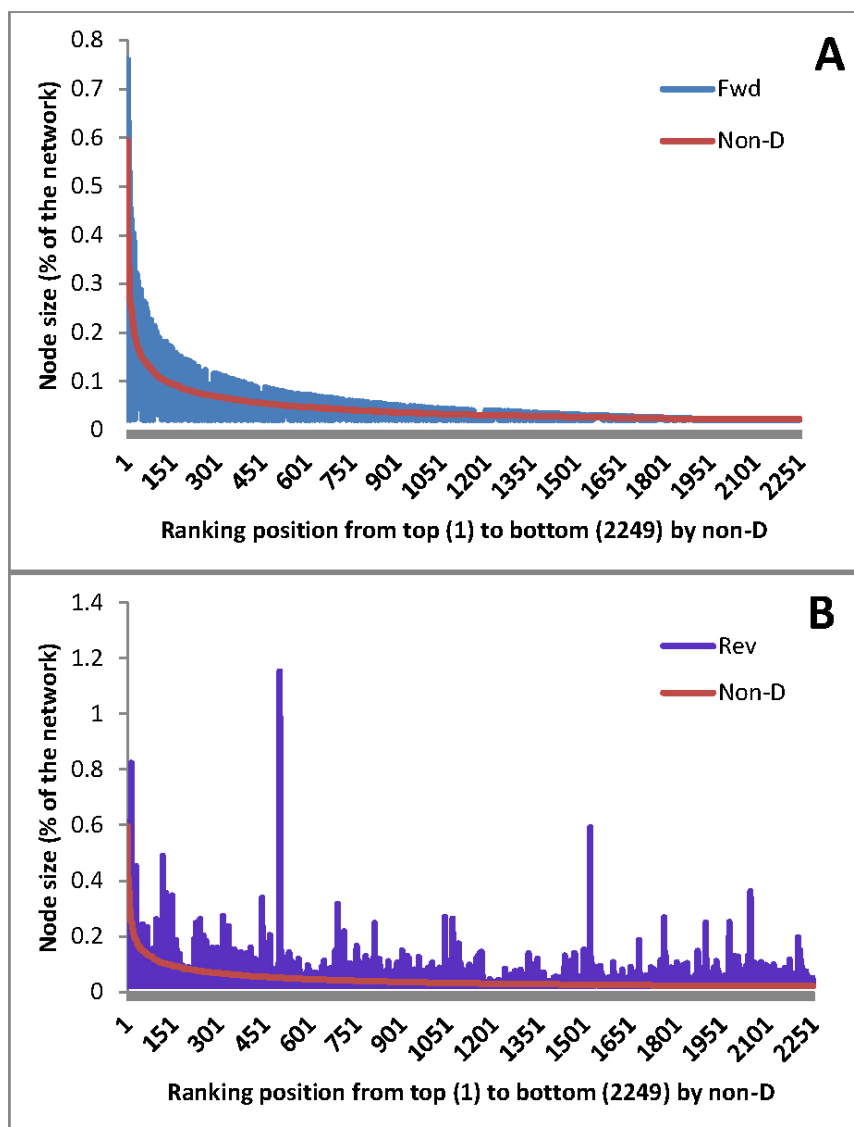
The proteins ranked in the top 50 by the forward, reverse, and non-directional rankings are mainly MAPK signaling pathway proteins (account for 35%, 30%, and 24%, respectively) and insulin signaling pathway proteins (account for 18%, 16%, and 20%, respectively) (Table 1) (Du et al., 2012). Those results suggest the overall importance of the MAPK and insulin signaling pathways for the cell. Interestingly, some of the lowest-ranking proteins are involved in the MAPK signaling pathway but not the insulin signaling pathway. All the insulin signaling pathway proteins are high-ranking or at least mid-ranking proteins.

The ubiquitin-protein ligase Casitas Blineage lymphoma (CBL) is ranked No. 1 in the reverse ranking, which means that CBL is the top-ranked regulator in the cell. This ligase is responsible for protein ubiquitination and is known to be involved in at least nine pathways, including the insulin signaling pathway (Figure 2). Mutation in the CBL gene has been implicated in a number of human cancers, including acute myeloid leukemia (Naramura et al., 2011). However, CBL-phosphoinositide 3-kinase (PI3K) interaction regulates differentiation and survival as well as bone resorption in osteoclasts, and the loss of CBL-PI3K interaction in mice prevents significant bone loss following ovariectomy (Adapala et al., 2014). Another top-ranked regulator is PTEN, which will be discussed later in the article.

Eight proteins are among the top50-ranked proteins in all three ranking approaches (forward, reverse, and non-directional) (Table 2). Those proteins are ACVR1, CDC42, RAC1, RAF1, RHOA, TGFBR1, TRAF2, and TRAF6 (see Table 2 for names). Each of these eight top-ranked proteins is involved in many pathways. Mutations of these eight proteins are implicated in many types of cancers and severe diseases.

### Human diseases caused by somatic mutations to the PI3K/PTEN/AKT pathway

Somatic mutation of insulin signaling pathway genes is often implicated in cancers or overgrowth syndromes, and there are numerous reports about this relationship in the literature, even in 2014 alone. For example, PTEN mutation is implicated in at least breast cancer (Kim et al., 2014; Pradella et al., 2014), colorectal cancer (Yu et al., 2014), lung cancer (Cumberbatch et al., 2014), and prostate cancer (Krohn et al., 2014). Mutation of the p110 $\alpha$  catalytic subunit of PI3K (PIK3Ca) was detected in various cancers, including breast cancer (Arsenic et al., 2014; Jelovac et al., 2014; Zardavas et al., 2014), intestinal cancer (Deming et al., 2014), and gastric cancer (Yang et al., 2014a). In addition, AKT E17K mutation is detected in common solid cancers and acute leukemias (Kim et al., 2008). Mutation of mechanistic target of rapamycin (mTOR), a junction between the MAPK signaling pathway and the insulin signaling pathway, is also implicated in various cancers (Grabiner et al., 2014). As well, the MAPK pathway is implicated in different malignancies (Kim et al., 2008). Polymorphism of DNA in tuberous sclerosis complex



**Figure 1.** Two views of the ion conduction pathway of GluA2 with either a glutamine (grey) or an arginine (blue) at codon 586. Structures are from pdb 3KG2 (Sobolevsky *et al.*, 2009). The view shows only the transmembrane spans and is from the inside looking out. As can be seen, the Q/R site sits directly in the ion permeation pathway. The Q/R substitution was generated with Pymol software. The position of the R side chain was not calculated via energy minimization, but merely reflects the most common rotamer for R.

**Table 1.** Mitogen-activated protein kinase (MAPK) signaling pathway and insulin signaling pathway proteins among the top-ranked and low-ranked proteins.

Ranking method	MAPK signaling/all proteins (%)	Insulin signaling/all proteins (%)
Forward top 50	35	18
Reverse top 50	30	16
Non-directional top 50	24	20
Forward bottom 50	6	0
Reverse bottom 50	10	0
Non-directional bottom 50	6	0

This table was adapted from Du et al. (2012). The percentages of insulin signaling pathway proteins were calculated from the supplementary files of Du et al. (2012).

**Table 2.** The eight proteins ranked in the top 50 by all three ranking methods—forward, reverse, and non-directional.

Protein name	Kegg ID	IPI number	Gene names	Definition	Pathway
ACVR1	hsa:90	IPI00029219	ACVR1, ACTRI, ACVR1A, ACVRLK2, ALK2, FOP, SKR1, TSRI	activin A receptor, type I	Cytokine–cytokine receptor interaction TGF (transforming growth factor)–beta signaling pathway
CDC42	hsa:998	IPI00007189	CDC42, CDC42Hs, G25K	cell division cycle 42 (GTP [guanosine 5'-triphosphate]–binding protein, 25 kDa)	MAPK (mitogen-activated protein kinase) signaling pathway Chemokine signaling pathway Endocytosis Axon guidance VEGF (vascular endothelial growth factor) signaling pathway Focal adhesion Adherens junction Tight junction T cell receptor signaling pathway Fc gamma Rmediated phagocytosis Leukocyte transendothelial migration Neurotrophin signaling pathway Regulation of actin cytoskeleton GnRH (gonadotropin-releasing hormone) signaling pathway Bacterial invasion of epithelial cells Epithelial cell signaling in <i>Helicobacter pylori</i> infection Pathogenic <i>Escherichia coli</i> infection Shigellosis <i>Salmonella</i> infection Pathways in cancer Renal cell carcinoma Pancreatic cancer
RAC1	hsa:5879	IPI00010271	RAC1, Rac-1, TC-25, p21-Rac1	ras-related C3 botulinum toxin substrate 1 (rho family, small GTP-binding protein Rac1)	MAPK signaling pathway Chemokine signaling pathway Phagosome Wnt signaling pathway Axon guidance VEGF signaling pathway Osteoclast differentiation Focal adhesion Adherens junction Toll-like receptor signaling pathway Natural killer cell–mediated cytotoxicity B cell receptor signaling pathway Fc epsilon RI signaling pathway Fc gamma Rmediated phagocytosis Leukocyte transendothelial migration Neurotrophin signaling pathway Regulation of actin cytoskeleton Pancreatic secretion Amyotrophic lateral sclerosis (ALS) Bacterial invasion of epithelial cells Epithelial cell signaling in <i>Helicobacter pylori</i> infection Shigellosis <i>Salmonella</i> infection Pathways in cancer Colorectal cancer Renal cell carcinoma Pancreatic cancer Viral myocarditis
RAF1	hsa:5894	IPI00021786	RAF1, CRAF, NS5, Raf-1, c-Raf	vraf1 murine leukemia viral oncogene homolog 1	MAPK signaling pathway ErbB signaling pathway Chemokine signaling pathway Vascular smooth muscle contraction VEGF signaling pathway Focal adhesion Gap junction Natural killer cell–mediated cytotoxicity T cell receptor signaling pathway B cell receptor signaling pathway Fc epsilon RI signaling pathway Fc gamma Rmediated phagocytosis Long-term potentiation Neurotrophin signaling pathway Serotonergic synapse Long-term depression Regulation of actin cytoskeleton Insulin signaling pathway GnRH signaling pathway Progesterone-mediated oocyte maturation Melanogenesis Tuberculosis Hepatitis C Influenza A Pathways in cancer Colorectal cancer Renal cell carcinoma Pancreatic cancer Endometrial cancer Glioma Prostate cancer Melanoma Bladder cancer Chronic myeloid leukemia Acute myeloid leukemia Non-small-cell lung cancer

RHOA	hsa:387	IPI00478231	RHOA, ARH12, ARHA, RHO12, RHOH12	ras homolog gene family, member A	Chemokine signaling pathway Endocytosis Vascular smooth muscle contraction Wnt signaling pathway TGF-beta signaling pathway Axon guidance Focal adhesion Adherens junction Tight junction T cell receptor signaling pathway Leukocyte transendothelial migration Neurotrophin signaling pathway Regulation of actin cytoskeleton Pancreatic secretion Bacterial invasion of epithelial cells Pathogenic <i>Escherichia coli</i> infection Pertussis Tuberculosis Pathways in cancer Colorectal cancer
TGFBR1	hsa:7046	IPI00005733	TGFBR1, AAT5, ACVRLK4, ALK-5, ALK5, LDS1A, LDS2A, MSSE, SKR4, TGFR-1	transforming growth factor-beta receptor 1	MAPK signaling pathway Cytokine-cytokine receptor interaction Endocytosis TGF-beta signaling pathway Osteoclast differentiation Adherens junction Chagas disease (American trypanosomiasis) HTLV1 (human Tlymphotropic virus type I) infection Pathways in cancer Colorectal cancer Pancreatic cancer Chronic myeloid leukemia
TRAF2	hsa:7186	IPI00030278	TRAF2, MGC: 45012, TRAP, TRAP3	TNF (tumor necrosis factor) receptor-associated factor 2	MAPK signaling pathway Protein processing in endoplasmic reticulum Apoptosis Osteoclast differentiation RIG1 (retinoic acid-inducible gene 1)-like receptor signaling pathway Adipocytokine signaling pathway Hepatitis C Herpes simplex infection Pathways in cancer Small-cell lung cancer
TRAF6	hsa:7189	IPI00743663	TRAF6, MGC: 3310, RNF85	TNF receptor-associated factor 6, E3 ubiquitin-protein ligase	MAPK signaling pathway Ubiquitin-mediated proteolysis Endocytosis Osteoclast differentiation Toll-like receptor signaling pathway NOD (nucleotide-binding oligomerization domain)-like receptor signaling pathway RIG1-like receptor signaling pathway Neurotrophin signaling pathway Pertussis Leishmaniasis Chagas disease (American trypanosomiasis) Toxoplasmosis Tuberculosis Hepatitis C Measles Herpes simplex infection Pathways in cancer Small-cell lung cancer

This table was adapted from Table S1 of Du et al. (2012).

(TSC), a junction between insulin signaling and MAPK signaling, is also reported to be implicated in cancers (Xiao et al., 1995).

However, insulin signaling pathway genes are also known to be implicated in various non-malignant overgrowth conditions, including LEOPARD syndrome (by PTPN11 [SHP2] mutations) (Edouard et al., 2010), Down syndrome (Follo et al., 2007; Troca-Marín et al., 2014), CLOVES (congenital lipomatous overgrowth, vascular malformations, epidermal nevis, spinal/skeletal anomalies/scoliosis) syndrome (Kurek et al., 2012; Keppler-Noreuil et al., 2014, 2015), and Proteus syndrome (Lindhurst et al., 2011, 2014; Marsh et al., 2011).

In addition to somatic mutations, animals and plants have also developmental and environmental variations (Li, 2009) and different types of variations, such as endopolyploidy and aneuploidy of the nuclear genome and stoichiometric

variations of the mitochondrial genome (Li, 2016). Little information is available, however, about the effects of these different types of somatic genome variation on pathway activities. At least, aneuploid variations, however, are expected to change the relative activities among genes and pathways.

#### **PTEN, PIK3Ca, and AKT—causal genes for cancer or non-cancer?**

Why does the mutation of the same genes (encoding PTEN, PIK3Ca, AKT, etc.) induce malignancy in many cases but non-cancer in others? One hypothesis is that differences in the activity of the protein and its pathways might be responsible for malignancy or non-malignancy (Kurek et al., 2012). Gene expression activity varies greatly depending on time, cellular physiological status, environmental factors, and even food. Differences in gene expression activity are likely not the only causal possibility for differences in malignancy and non-malignancy. It was

reported that mutation in both the insulin pathway and the MAPK pathway can have more severe consequences for the cell. Both AKT (insulin signaling pathway) and extracellular-signal-regulated kinase (ERK) (MAPK signaling pathway) phosphorylate distinct sites on TSC2, and thus its Rheb GTPase-activating protein (GAP) activity is more repressed and, consequently, the stimulation of mTORC1 signaling is magnified, in comparison with the effect of either input alone (Winter et al., 2011). MicroRNA (miRNA) is known to be implicated in regulating the insulin signaling pathway genes, particularly PTEN and sometimes PIK3Ca, in various types of cancers (Fang et al., 2014; Geng et al., 2014; Ma et al., 2014a, 2014b; Wang et al., 2014; Yang et al., 2014b; Zhu et al., 2014). In most cases, miRNA is encoded unusually by other genes, not the targeted gene itself. In addition to "cancer genes", these same miRNA may also target other genes. Cell migration, critical in cancer progression, is known to be promoted by lysophospholipids (Panetti and Mosher, 2000; Monet et al., 2009) and, in certain cases, also requires very active mitochondrial fission (Han et al., 2015). The stimulation of cancer cell migration by lysophospholipids occurs through activation of the TRPV2 (transient receptor potential cation channel subfamily V, member 2) channel (Monet et al., 2009). Editing of RNA is known to act on many messenger RNAs (mRNAs) of channel and transporter genes and affects the translated products (Holmgren and Rosenthal, 2015). A miRNA known as miR429 was found to inhibit migration and invasion of breast cancer cells *in vitro* (Ye et al., 2015). Another miRNA, miR153, was found to inhibit cell migration and invasion of human non-small-cell lung cancer by targeting the three-prime untranslated region (3'UTR) of ADAM19 (A disintegrin and metalloproteinase 19) gene transcripts (Shan et al., 2015). The 3'UTR region of genes is known to have conserved motifs and characteristics that affect mRNA polyadenylation (Li, 2014; Li and Du, 2014). The targeting of ADAM19 transcripts by miR153 may suggest that ADAM19 gene activity can be changed through regulation of mRNA polyadenylation and mRNA stability. If cell migration plays a critical role in cancers, why did many studies actually find PTEN, PIK3Ca, AKT, CBL, or MAPK genes to be likely cancer genes? Are cancers the result of somatic overactivation in both a cell proliferation signal transduction gene and a cell migration gene? Further research is required to clarify why mutation of PTEN, PIK3Ca, AKT, and various other signaling genes causes cancers sometimes but non-malignant tumors or just overgrowths at other times. Are there other genes or molecular mechanisms that are more responsible or jointly responsible for determining whether a condition is malignant or non-malignant?

#### **PTEN/AKT activity, food nutrition, growth factor, and fat accumulation**

Glutamine supply is known to stimulate the activity of the AKT/mTOR pathway (Lambertucci et al., 2012; Modi et al., 2014) and increase protein synthesis (Lambertucci et al., 2012). Gene expression analysis suggests that PIK3Ca mutation can upregulate glycolysis as well as affect other pathways such as glutaminolysis (Foster et al., 2012). Nutrient dependency studies of PIK3Ca mutant cells

revealed that the growth of *PIK3Ca* mutant cells is highly dependent on glucose, whereas glutamine dependency is likely independent of PIK3Ca status (Foster et al., 2012). Although further research is required to clarify how glutamine and glucose regulate the AKT/mTOR pathway, it is convincing that nutritional supply affects the activity of metabolic pathways and the PI3K/AKT/mTOR signaling pathway, which is known to be deeply involved in cell growth.

Phosphatidylinositol (3,4,5)trisphosphate (PIP3) promotes AKT activity, and AKT then phosphorylates TSC2, recruits protein 1433 to TSC2, and dissociates the TSC1/TSC2 dimer. That dissociation causes the loss of GAP activity from TSC2 and renders TSC2 unable to hydrolyze Rheb-GTP (guanosine-5'-triphosphate) to Rheb-GDP (guanosine-5'-diphosphate). Therefore, Rheb-GTP can activate mTOR and consequently protein synthesis and cell proliferation (Mendoza et al., 2011) (Figure 2). For its part, PI3K converts phosphatidylinositol (3,4)bisphosphate (PIP2) to PIP3 and promotes AKT/mTOR activity and cell growth. Activation mutations of PI3K or AKT are involved in cancer and overgrowth conditions. Moreover, PTEN converts PIP3 to PIP2, reduces the PIP2 concentration, and therefore plays a role as a tumor suppressor or overgrowth suppressor. It was reported that obesity promoted by high fat uptake occurs through the PI3K signaling pathway (Klöckener et al., 2011; Ribeiro et al., 2012). Fasting can decrease PI3K/AKT pathway activity in nutrition-regulated lipolysis in rainbow trout (*Oncorhynchus mykiss*) (Bergan et al., 2012). Both the insulin-like growth factor I (IGF1)/PI3K/AKT and IGF1/MAPK/ERK pathways *in vivo* in skeletal muscle are regulated by nutrition and contribute to somatic growth in fine flounder (*Paralichthys adspersus*) (Fuentes et al., 2011).

A long-term oversupply of food (eventually converted mainly to glucose) may cause a long-term high content of inulin and high activity of the insulin/PI3K/PTEN/mTOR signaling pathway, resulting in weight gain or sometimes increasing the risk of diabetic issues if this insulin signaling pathway becomes insensitive. An oversupply of phospholipid-rich fatty food might increase the overall supply of PIP2 or PIP3, two relatively rare forms of phospholipids. Further research is required to determine whether this overall increase in PIP2 and PIP3 enhances the activity of the PIP2–PIP3 conversion cycle, results in a general increase in PIP3, and promotes cell growth (Figure 2). Mutated cells with PTEN/PI3K pathway activation with an increase in PIP3 may signal the cells to be more aggressive in consuming food nutritional supply (glucose, glutamine, and phospholipids) than are the non-mutated cells in the chimeric tissues. Cells that have PTEN deficiency or PIK3CA over-activation are likely more competitive than normal cells in terms of the use of nutrients. PTEN/PIK3CA somatic mutation patients probably should avoid over-fasting and overeating. Further research is required to clarify the effects of food on the growth of different tumors and to determine how to manage food intake for patients on a case-by-case basis. In addition, PIK3CA/AKT pathway patients since various growth factors (such as insulin-like growth factors IGF-I

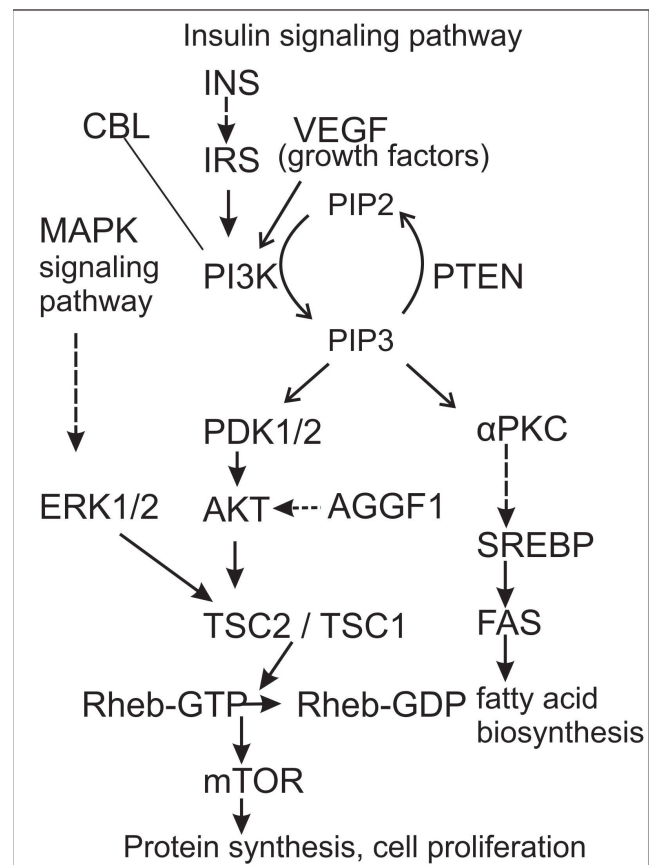
and vascular endothelial growth factors) can enhance the insulin signaling and PIK3CA/AKT pathway (Figure 2). In a study for people in the United Kingdom, compared with meat-based diet, a plant-based diet is associated with lower circulating levels of total IGF-I and likely also a lower risk of cancer (Allen et al. 2002). We suggest that food type and intake amount should be appropriately managed with close monitoring of the effects of that management for PI3K/AKT pathway patients.

Several overgrowth syndromes, such as Proteus (Cohen Jr., 1993, 2005, 2014), CLOVES (Alomari, 2009; Klein et al., 2012), and likely also Klippel-Trenaunay (Ashkan and Moore, 2002; Kihiczak et al., 2006) and PROS (PIK3CA-related overgrowth spectrum) (Keppler-Noreuil et al., 2015), share a number of common symptoms, and for many years it was challenging to clearly distinguish between those syndromes. Now, molecular research has found that the genes likely responsible for those overgrowth syndromes are in the same interrelated pathways. The protein encoded by the genes responsible for Proteus syndrome is AKT (Lindhurst et al., 2011; Marsh et al., 2011), for PROS syndrome is PIK3Ca (Kurek et al., 2012; Keppler-Noreuil et al., 2015), and for Klippel-Trenaunay syndrome is angiogenic factor with G patch and FHA domains 1 (AGGF1) (Kihiczak et al., 2006; Chen et al., 2013) (Figure 2).

Lipomas are common in various overgrowth syndromes, such as Proteus (Taghinia et al., 2007; Nakayama et al., 2009), CLOVES (Alomari, 2009), and likely also Klippel-Trenaunay (Ashkan and Moore, 2002; Klein et al., 2012). However, a truncal lipomatous mass is reported to be one of the uniform and highly characteristic features of CLOVES syndrome (Alomari, 2009), as are multiple lipomas for hemihyperplasia–multiple lipomatosis (HHML) syndrome (Biesecker et al., 1998). A lipomatous mass is not reported to be as highly characteristic in Klippel-Trenaunay syndrome. To date, the molecular genetic mechanism for this likely difference between these syndromes is not clear, and it is also not clear why these conditions often have a lipomatous mass.

In this article, we tentatively suggest that the protein kinase C (PKC)/sterol regulatory element-binding protein (SREBP)/fatty acid synthase (FAS) pathway is involved in the accumulation of lipomatous tissue in a mass. As illustrated in Figure 2, one of the partitioning pathways for PIP3 is the PKC/SREBP/FAS pathway, which leads to fatty acid synthesis. Protein kinase C can be activated by PIP3 (Singh et al., 1993). Alpha PKC at least partly regulates hepatic SREBP1c, which controls lipid synthesis (Sajan et al., 2009). The likely causal gene for HHML and CLOVES syndromes encodes PIK3Ca, the catalytic subunit of PI3K, at a relatively upstream step of the pathway. CLOVES syndrome likely increases PIP3, which likely stimulates the activity of the PKC/SREBP/FAS pathway and causes the growth of a lipomatous mass (Figure 2). Given that the implicated gene for Proteus syndrome is AKT, which happens at a relatively downstream step (Figure 2), this syndrome is expected to have a lesser degree of lipomatous mass than CLOVES syndrome does. The

causal gene for Klippel-Trenaunay syndrome in some patients encodes for AGGF1, which is even farther downstream (indirectly involved in the pathway) and is therefore expected to cause even less lipoma than Proteus syndrome does (Figure 2). It is also known that somatic *PIK3CA* mutations are the most common cause of Klippel-Trenaunay syndrome and other lymphatic malformations (Luks et al., 2015). It is likely that Klippel-Trenaunay syndrome is not sufficiently defined at the symptom level and its clinical distinction from other lymphatic malformation syndromes is sometimes difficult. Some patients of this syndrome may have somatic mutation in *AGGF1*, some in *PIK3CA*, and some may have mutations in both genes. A combination between gene analysis and clinical diagnostics may identify the symptom differences among these three groups. According to the position of *AGGF1* in the signaling pathway presented in Figure 2, we speculate that Klippel-Trenaunay syndrome patients who have somatic mutations in *AGGF1* but not in *PIK3CA* may have less degree of lipomatous mass. Further research is required to confirm our hypothesis (using the position difference for the causal genes in the PI3K/AKT pathway and PKC/SREBP partitioning of PIP3) for the degree of difference in lipomatous mass among CLOVES, Proteus, and Klippel-Trenaunay syndromes.



**Figure 2.** Joint action of the MAPK and insulin signaling pathways on cell proliferation and a potential pathway for PIP3 partitioning to fat accumulation. For the meanings of the abbreviations, see the abbreviations list at the beginning of the article.



### Insulin signaling pathway in plants

Plants have insulin (or glucokinin), and the insulin signaling pathway likely regulates maize (*Zea mays*) growth (García Flores et al., 2001; Xavier-Filho et al., 2003; Dinkova et al., 2007). Plant insulin can also activate maize ribosomal S6 protein kinase (ZmS6K) in germinating maize axes, and that activity can be inhibited by rapamycin, indicating that it depends on mTOR activity (Dinkova et al., 2007). Overall, however, the insulin signaling pathway and its function have been studied much less in plants. Gene knockout approaches may be able to clarify whether plant insulin and its signaling system play a critical role in regulating plant growth. Further research is required to understand whether some herbal products such as Chinese herbal medicines that are used in treating diabetes actually have more endogenous insulin.

### MAPK signaling pathway and plant stress tolerance

Current annotations of protein families predict approximately 1053 kinases in *Arabidopsis* (Peck, 2006) and 1429 kinases in rice (*Oryza sativa*) (Dardick et al., 2007). The large pool of kinases indicates the importance of phosphorylation mechanisms in the growth and development of plants. Protein kinases can also form a cascade to process signals much more efficiently. One such case is the MAPK cascade (Xing and Laroche, 2011; Hamel et al., 2012). All eukaryotes have four classes of functionally related kinases that appear as gene families, and these proteins are organized in hierarchical fashion in a cascade. These include mitogen-activated protein kinase kinase kinase (MAPK<sup>KKK</sup>), mitogen-activated protein kinase kinase (MAPK<sup>KK</sup>), mitogen-activated protein kinase (MAPK), and MAPK.

A recent PageRank analysis of the human protein interactome has put MAPKs as some of the top-ranked proteins together with some other members of signaling pathways (Du et al., 2012). The top forward ranking indicates that MAPKs are very active receivers of signals, and the top reverse ranking indicates that some MAPKs are very active regulators or signal emitters to other proteins. Several MAPKs are top-ranked in both forward and reverse ranking, suggesting that these proteins themselves are both active signal receivers and active regulators, which fits well with the cascade model of the MAPK pathway (Xing et al., 2002; Cvetkovska et al., 2005).

Cascades of MAPKs could be regulated by diverse stimuli such as pathogen attacks, wounding, high or cold temperatures, high salinity, ultraviolet radiation, ozone, reactive oxygen species, drought, and high osmolarity (Cvetkovska et al., 2005; Tena et al., 2011). When an upstream MAPK<sup>KKK</sup> or MAPK<sup>KK</sup> is activated by external stimuli, it activates downstream MAPKs through phosphorylation on serine and threonine residues in a conserved S/T-X3-5-S/T motif, and then the MAPKs activate downstream MAPKs through phosphorylation on threonine and tyrosine residues in the TXY motif (Stulemeijer et al., 2007). The activated MAPKs will phosphorylate a variety of substrates, including transcription factors, other protein kinases, enzymes, and

cytoskeleton-associated proteins (Xing et al., 2002; Xing and Laroche, 2011).

In particular, tMEK2 (also named LeMKK2) is a known MAPKK in tomato and has been shown to regulate the expression of antifungal factors, including beta-1,3-glucanase and endochitinase, in response to pathogen attacks (Xing et al. 2003). A constitutively activated tomato MAPKK gene, *tMEK2<sup>MUT</sup>*, was created, in which the tMEK2 was constitutively activated by replacing amino acids S221 and T226 between subdomains VII and VIII with glutamic acid (Xing et al., 2003). When overexpressed, *tMEK2<sup>MUT</sup>* was found to increase resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* in tomato (*Solanum lycopersicum*) (Xing et al. 2003) and the fungal pathogen *Puccinia triticina* in wheat (*Triticum aestivum*) (Xing et al., 2003; Fan et al., 2009). As well, gloxinia (*Sinningia speciosa*) plants overexpressing *tMEK2<sup>MUT</sup>* had smaller leaves, increased contents of chlorophyll in leaves, and better recoveries of plant growth after cold or heat stresses in comparison with the controls, which were wild-type plants (Sun et al., 2012).

The identification and analysis of specific kinase substrates in any kinase pathway represent a major challenge in the study of signaling events. Although we identified hundreds to thousands of phosphopeptides in a single experiment, the functional significance of many phosphorylation sites remains unclear, and most of the identified phosphoproteins have not been analyzed in terms of their role in any specific plant developmental processes or plant response networks. Proteomic approaches have been applied to monitor immediate downstream components of specific defense pathways. Two-dimensional gel electrophoresis (2DE) and liquid chromatography–tandem mass spectrometry (LC-MS/MS) were used to compare wild-type tomatoes and transgenic tomato carrying *tMEK2<sup>MUT</sup>*, and the analysis revealed a group of phosphoproteins in *tMEK2<sup>MUT</sup>*-transgenic tomato plants (Thurston et al. 2005). The nucleotide diphosphate protein kinase TAB2, which has long been considered a non-regulatory housekeeping enzyme, is of particular interest (Kimura et al., 2003). It was found that TAB2 was upregulated by the overexpression of *tMEK2<sup>MUT</sup>* in tomato protoplasts and plants (Xing et al., 2008). However, a mutation at the TAB2 kinase interaction motif was found to significantly reduce the *tMEK2<sup>MUT</sup>*-induced upregulation of the PR1b1, beta-1,3-glucanase, and chitinase genes (Xing et al., 2008). Protein pull-down assays suggested an interaction between TAB2 and LeMPK3 but not between TAB2 and other members of tomato MAPKs (Xing et al., 2008). Overexpression of the wild-type TAB2 was also found to enhance resistance to virulent *Pseudomonas syringae* pv. *tomato* (Xing et al., 2008). Thanks to a phosphoproteomics approach in the enhanced tMEK2 defense pathway, TAB2 has been identified as a downstream protein of LeMPK3 as well as an effective pathway component in disease resistance mediated by tMEK2 (Xing and Laroche 2011).

Salt is a significant source of stress that affects plants from germination through to seed development (Bressan et al.,

2009). The stress response kinase AtMKK1 was found in some studies to be capable of activating the MAPK proteins AtMPK3, AtMPK4, and AtMPK6 (Colcombet and Hirt, 2008). One study found that AtMKK1-knockout *Arabidopsis* plants were more tolerant of salt during both germination and adulthood (Conroy et al., 2013). According to proteomic analysis, the levels of the alpha subunit of mitochondrial H<sup>+</sup>-ATPase, mitochondrial reduced nicotinamide adenine dinucleotide (NADH) dehydrogenase, and mitochondrial formate dehydrogenase (FDH) were enhanced in the AtMKK1-knockout plants under high salinity stress (Conroy et al., 2013). The increase in FDH may suggest the possible involvement of this enzyme in salt tolerance, particularly in light of its impact on drought (Herman et al., 2002). Interestingly, a negative correlation between AtMKK1 and AtFDH (*Arabidopsis thaliana* FDH) also seemed to be suggested by gene coexpression analysis (Conroy et al., 2013). It is unclear whether other pathways were activated by the knockout plants during salt treatment, in which the AtMKK1 pathway was blocked; those pathways could have not only increased the FDH level but also resulted in other changes in these plants, potentially contributing to salt tolerance (Conroy et al. 2013).

The MAPK proteins AtMPK3 and AtMPK6 were also studied in a different system. When *Medicago sativa* stress-induced MAPKK (SIMKK) was overexpressed in *Arabidopsis*, AtMPK3 and AtMPK6 kinases were found to be salt insensitive during seed germination but highly sensitive to salt stress at the seedling stage (Ovečka et al., 2014). Proteomic analysis of plants that overexpressed SIMKK–yellow fluorescent protein (YFP) indicated that proteins directly or indirectly involved in salt stress responses (including catalase, peroxiredoxin, glutathione S-transferase, nucleoside diphosphate protein kinase 1, endoplasmic reticulum luminal-binding protein 2, and plasma membrane aquaporins) were differentially regulated (Ovečka et al., 2014). In *Arabidopsis* there are 20 MAPKs, 10 MAPKKs, and 60 MAPKKKs (MAPK Group, 2002). Because of those large numbers, the specificity of protein–protein interactions, the complex signal filtering and amplification processes, and most importantly their differential involvement in multiple activities of plant development and plant responses to internal and external signals (e.g., cell division vs. stress response), it is clear that caution should be exercised when assessing manipulation strategies, so that the manipulations will achieve the desired results without having detrimental effects on plant growth and development.

### Protein domain evolution and potato type II proteinase inhibitors

Proteins interact chiefly at the domain or motif level, and there are likely over a million instances of peptide motifs in the human proteome (Tompa et al., 2014). Where do these domains come from? In plants, the generation of sequence diversity is likely a defense strategy to confine insect attack (Mishra et al., 2012). Phylogenetic frameworks and residue contact networks in protein families may be helpful for studying the evolution that leads to structural changes (Zhang et al., 2013). In one study, when a C-terminal serine

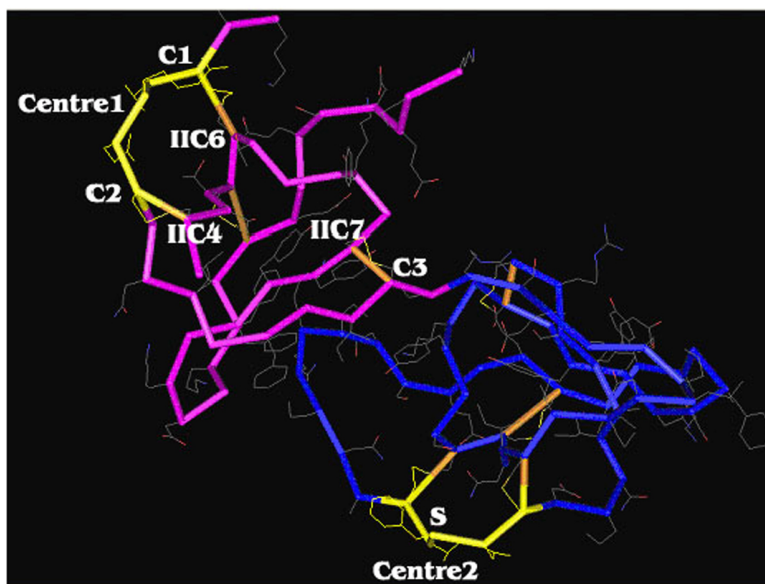
residue proximal to the inter-chain disulfide bond of a human immunoglobulin 1 lambda light chain was removed, enhanced antibody stability and antibody-dependent cell-mediated cytotoxicity were mediated (Shen et al., 2013). It is likely that a Pill without two conserved disulfide bonds is so remarkably efficient because the reactive site loop has enhanced flexibility and hydrogen-bond density (Joshi et al., 2014).

It is obviously very challenging to figure out the evolutionary path and estimate the functional status of the intermediate versions of domains and genes during the emergence of novel domains. Gene families with domain variants can be very helpful for studying protein domain evolution. Li et al. (2011) were able to conclude that the intermediate versions during the emergence of a novel potato Pill must be functional without a pseudogene stage.

The most common type II domain is double-headed in shape and usually has two active sites with one on each subdomain (head). Each subdomain has eight conserved disulfide bonds, with two disulfide bonds on each active site. However, in a group of potato Pills, Li et al. (2011) found that one or both of the onsite disulfide bonds are absent (Figure 3). These cysteine residues directly pair to form the disulfide bond(s) at the protein structure level but are distantly separated at the primary sequence level (Figure 4). Since mutation must happen at the DNA sequence level in the genes, this loss of the cysteine codons in the two separated sequence places must happen in different events. The selective loss of the bond-pairing cysteines suggests that, after one cysteine is lost, the remaining cysteine in the bond must also be eliminated in order to ensure that the mutated domain has a beneficial function for the plant. This selective elimination of the second cysteine in the pair is evidence of a response to natural selection or breeding selection. To respond to selection, the intermediate versions of the novel proteinase inhibitor domain must be functional, without a pseudogene stage.

### Concluding remarks and outlook

High-ranked proteins, also called control proteins, in the human signal flow protein network are likely to be functionally very important proteins, because they are involved in many signaling and other pathways and their mutation is usually implicated in many diseases. In the human protein network, signal emission and signal reception are systematically different in terms of correlation with the degree of nodes and cellular locations. Proteins of the MAPK and insulin signaling pathways are among the top-ranked proteins. Proteins such as PTEN, PI3K, and AKT in the insulin signaling pathway are frequently implicated in cancers and overgrowth conditions. We propose a hypothesis that PIP3 partitioning by a PKC/SREBP/FAS pathway may explain the degree of difference in lipomatous mass between various overgrowth syndromes. We also suggest that food nutrition be appropriately managed for PTEN/PI3K/AKT pathway patients in order to reduce tumor growth. Manipulation of, or screening for mutants of, the MAPK pathway can genetically improve plant tolerance to various biotic and



**Figure 3.** A type II proteinase inhibitor structure showing the missing disulfide bonds (C1-IIC6 and C2-IIC4) at active site 1 (Centre1) in a group of potato type II proteinase inhibitors. Source: Li et al. (2011).

**A. Primary sequence of the 1<sup>st</sup> sub-domain**

Cysteine:	1	2	3	4	56	7	8	
<b>PiII-TM1</b>	CEGESDPKRPNA	<b>CTFNC</b>	DPNIAYSR	CPRSQGKSLIYPTG	CTT	<b>CCTGYKG</b>	CYYFGKDGKVF	CEGESDE
<b>PiII-TM2</b>	YLLVSTVEHANAC	<b>CTKEC</b>	GNLGYGI	CPGSEGS--PENPI	CTN	<b>CCSGYKG</b>	CNYYYANGTFI	CEGTSDP
<b>PiII-NA</b>	CEGESDPRNPKA	<b>CTLNC</b>	DPRIAYGV	CPRSEEK--KNDRI	CTN	<b>CCAGTKG</b>	CKYFSDDGTFV	CEGESDP
<b>PiII-NT</b>	CEGSSDPKPNV	<b>CPQFC</b>	DPDIAYSK	CPRSEGETIINPTG	CTT	<b>CCTGYKG</b>	CYYFGQDGEFV	CEGESDE
<b>PiII-CA</b>	CEGESDPNPKP	<b>CTLNC</b>	DPRIFYSK	CPRSEGN--AENRI	CTN	<b>CCAGRKG</b>	CNYYSADGTFI	CEGESDP
<b>PiII-ST</b>	LVSAMEHVDAKAC	<b>CTLEC</b>	GNLGFGI	CPRSEGS--PENRI	CTN	<b>CCAGYKG</b>	CNYYSANGAFI	CEGESDP
-----								
<b>ABRPI-TM</b>	GMILLASDFEHA--KA	<b>CTKEC</b>	DTRIDFGI	CPLLETK--RVEGL	CTN	<b>CCAGKKG</b>	CKYFSKDGTYI	CDGSEW
<b>Pi6C-Ke</b>	GMILLASDFEHADAKA	<b>WTKES</b>	DRRIDYGI	CYPYLGTK-KVGAPL	CVN	<b>CCSGKIG</b>	CKYFNKDGTFI	CDGGER
<b>Pi7Ca-DC</b>	GMILLASDFEH	<b>ADAKE</b>	DPRIDFGI	CYPYLGTK--KVDGI	CIN	<b>CCSGYKE</b>	CKYFSKDYTFI	CDGESKD
<b>Pi7Cb-Sh</b>	GMILLASDFEH	<b>ADAKE</b>	DPRIDFGI	CYPYLGTK--KVDGI	CIN	<b>CCSGYKE</b>	CKYFSKDYTFI	CDGESTD
<b>Pi7Cc-Sh</b>	GMILLASDFEH	<b>ANAKK</b>	DPRIDFGI	CYPYLGTK--KVDGI	CIN	<b>CCSGFKE</b>	CKYFSKDYTFI	CDGESKD
<b>Pi7Cc-Ke</b>	GMILLASDFEH	<b>ANAKK</b>	DPRIDFGI	CYPYLGTK--KVDGI	CIN	<b>CCSGFKE</b>	CKYFSKDYTFI	CDGESKD
<b>Pi7Cd-ke</b>	GMILLASDFEH	<b>ANAKK</b>	DPRIDFGI	CYPYLGTK--KVDGI	CIN	<b>CCSGFKE</b>	CKYFSKDYTFI	CDGESKD

**B. Primary sequence of the 2<sup>nd</sup> sub-domain**

Cysteine:	1	2	3	4	56	7	8		
<b>PiII-TM1</b>	EGESDPKRPNA	<b>CTFNC</b>	DPNIAYSR	CPRSQGKSLIYPTG	CTT	<b>CCTGYKG</b>	CYYFGKDGKVF	CEGESDEPKAN	
<b>PiII-TM2</b>	VCEGESIEPKG	<b>CTKEC</b>	DPRVAYMT	CPSSGLA--KLNQV	CVN	<b>CCSAGEG</b>	CKLYDNDGSLL	CTGEPQSISTA	
<b>PiII-NA</b>	EGESDPRNPKA	<b>CTLNC</b>	DPRIAYGV	CPRSEEK--KNDRI	CTN	<b>CCAGTKG</b>	CKYFSDDGTFV	CEGESDPRNPK	
<b>PiII-NT</b>	VCEGESDEPKS	<b>CTTEC</b>	DPRVATIS	CPFSGLV--KINQE	CIN	<b>CCNADKG</b>	CELYDNDGSLI	CTGGEPQSA--	
<b>PiII-CA</b>	EGESDPNPKP	<b>CTLNC</b>	DPRIFYSK	CPRSEGN--AENRI	CTN	<b>CCAGRKG</b>	CNYYSADGTFI	CEGESDPNPKP	
<b>PiII-ST</b>	EGESDPKPKA	<b>CPRNC</b>	DPHIAYSK	CPRSEGKSLIYPTG	CTT	<b>CCTGYKG</b>	CYYFGKNGKVF	CEGESDEPKAN	
-----									
<b>ARP-TM</b>	VSEKNNL	<b>KKA</b>	CTKEC	DTRIDFGI	CPLLETK--RVEGL	CTN	<b>CCAGKKG</b>	CKYFSKDGTYI	CDGESEWVSEK
<b>Pi6C-Ke</b>	DGGSER	<b>DGK</b>	CTKEC	DPRIDFGI	GFCAYVETK--IVNGL	STN	<b>CSAGKKG</b>	CKYFSNDGTYI	CDGESEWVSEK
<b>Pi7Ca-DC</b>	GESKDW	<b>QSR</b>	CSKE	CDRRIEFGI	CVTDLTK--TVNGL	CTT	<b>CSAGKKG</b>	CKYFNNNGTYI	CDGEYEWLSEG
<b>Pi7Cb-Sh</b>	GESTDW	<b>QSR</b>	CSKE	CDRRIEFGI	CVTDLTK--TVNGL	CTT	<b>CSAGKKG</b>	CKYFNNNGTYI	CDGEYEWLSEG
<b>Pi7Cc-Sh</b>	GESKDW	<b>ESR</b>	CSKE	CDRRIEFGI	CVTDLTK--TVNGL	CTT	<b>CSAGKKG</b>	CKYFNNNGTYI	CDGEYEWLSEG
<b>Pi7Cc-Ke</b>	GESKDW	<b>ESR</b>	CSKE	CDRRIEFGI	CVTDLTK--TVNGL	CTT	<b>CSAGKKG</b>	CKYFNNNGTYI	CDGEYEWLSEG
<b>Pi7Cd-Ke</b>	GESKDW	<b>QSR</b>	CSKE	CDRRIEFGI	CVTDLTK--TVNGL	CTT	<b>CSAGKKG</b>	CKYFNNNGTYI	CDGEYEWLSEG

**Figure 4.** Primary sequence of potato proteinase inhibitor type II (PiII) subdomains, showing the selective loss of paired partners of one or two disulfide bonds, although the partners are distant at the primary sequence level. Note that cysteine 1 in Panel A pairs with cysteine 6 in Panel B to form a disulfide bond (See "C1-IIC6" in Figure 3). When one of the cysteines is lost, the other member of the pair is also simultaneously lost (e.g., the loss of the C1-IIC6 pair in Pi7C proteins and both the C1-IIC6 and C2-IIC4 pairs in Pi6C-Ke. See Figure 3 for the reference structure), suggesting that the intermediate versions during domain evolution were functional and reacted to selection. Source: Li et al. (2011).

abiotic stresses. Novel protein domains, at least in some proteinase inhibitors, evolved from existing ones, and the intermediate versions of the domains were likely functional and responded to selection. Further research is required to understand why signal emission and signal reception systems are different, which sites to target for the high-ranked proteins in drug discovery, how to manage food nutrition for signaling pathway patients, whether the PKC/SREBP pathway is indeed responsible for lipomatous masses in PTEN/PI3K/AKT pathway patients, and whether it is the upstream PTEN and PI3K or the mTOR (by rapamycin) that should be targeted in treatment. Further research is also required to characterize the function of the PI3K/AKT/mTOR pathway in plants, to screen for higher-yield somatic mutants of the pathway in vegetatively propagated plants, and to investigate how to manipulate the pathway for healthier food and more-productive crops.

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### References

- Adapala, N.S., Holland, D., Scanlon, V., Barbe, M.F., Langdon, W.Y., Tsygankov, A.Y., Lorenzo, J.A., and Sanjay, A. (2014). Loss of Cbl-PI3K interaction in mice prevents significant bone loss following ovariectomy. *Bone* 67, 1-9.
- Allen, N.E., Appleby, P.N., Davey, G.K., Kaaks, R., Rinaldi, S., and Key, T.J. (2002). The associations of diet with serum insulin-like growth factor I and its main binding proteins in 292 women meat-eaters, vegetarians, and vegans. *Cancer Epidemiol Biomarkers Prev* 11, 1441-1448.
- Alomari, A.I. (2009). Characterization of a distinct syndrome that associates complex truncal overgrowth, vascular, and acral anomalies: A descriptive study of 18 cases of CLOVES syndrome. *Clin Dysmorphol* 18, 1-7.
- Arsenic, R., Lehmann, A., Budczies, J., Koch, I., Prinzler, J., Kleine-Tebbe, A., Schewe, C., Loibl, S., Dietel, M., and Denkert, C. (2014). Analysis of PIK3CA mutations in breast cancer subtypes. *Appl Immunohistochem Mol Morphol* 22, 50-56.
- Ashkan, K., and Moore, A.J. (2002). Spinal cord compression caused by an extradural lipoma in Klippel-Trenaunay-Weber syndrome. Case illustration. *J Neurosurg* 97, 269.
- Bergan, H.E., Kittilson, J.D., and Sheridan, M.A. (2012). Nutrition-regulated lipolysis in rainbow trout (*Oncorhynchus mykiss*) is associated with alterations in the ERK, PI3K-Akt, JAK-STAT, and PKC signaling pathways. *Gen Comp Endocrinol* 176, 367-376.
- Bhattacharyya, R.P., Reményi, A., Yeh, B.J., and Lim, W.A. (2006). Domains, motifs, and scaffolds: The role of modular interactions in the evolution and wiring of cell signaling circuits. *Annu Rev Biochem* 75, 655-680.
- Biesecker, L.G., Peters, K.F., Darling, T.N., Choyke, P., Hill, S., Schimke, N., Cunningham, M., Meltzer, P., and Cohen Jr., M.M. (1998). Clinical differentiation between Proteus syndrome and hemihyperplasia: Description of a distinct form of hemihyperplasia. *Am J Med Genet* 79, 311-318.
- Bressan, R., Bohnert, H., and Zhu, J.-K. (2009). Perspective: Abiotic stress tolerance: From gene discovery in model organisms to crop improvement. *Mol Plant* 2, 1-2.
- Brin, S., and Page, L. (1998). The anatomy of a large-scale hypertextual Web search engine. *Comput Netw and ISDN Syst* 30, 107-117.
- Cao, M., Pietras, C.M., Feng, X., Doroschak, K.J., Schaffner, T., Park, J., Zhang, H., Cowen, L.J., and Hescott, B.J. (2014). New directions for diffusion-based network prediction of protein function: Incorporating pathways with confidence. *Bioinformatics* 30, i219-i227.
- Chen, D., Li, L., Tu, X., Yin, Z., and Wang, Q. (2013). Functional characterization of Klippel-Trenaunay syndrome gene AGGF1 identifies a novel angiogenic signaling pathway for specification of vein differentiation and angiogenesis during embryogenesis. *Hum Mol Genet* 22, 963-976.
- Chen, Z., Yang, H., Ma, J., Lei, J., and Gao, H. (2011). Time-based query classification and its application for page rank. *J Comput Inf Syst* 7, 3149-3156.
- Cohen Jr., M.M. (1993). Proteus syndrome: Clinical evidence for somatic mosaicism and selective review. *Am J Med Genet* 47, 645-652.
- Cohen Jr., M.M. (2005). Proteus syndrome: An update. *Am J Med Genet C Semin Med Genet* 137C, 38-52.
- Cohen Jr., M.M. (2014). Proteus syndrome review: Molecular, clinical, and pathologic features. *Clin Genet* 85, 111-119.
- Colcombet, J., and Hirt, H. (2008). *Arabidopsis* MAPKs: A complex signalling network involved in multiple biological processes. *Biochem J* 413, 217-226.
- Conroy, C., Ching, J., Gao, Y., Wang, X., Rampitsch, C., and Xing, T. (2013). Knockout of AtMKK1 enhances salt tolerance and modifies metabolic activities in *Arabidopsis*. *Plant Signal Behav* 8, e24206.
- Csermely, P., Korcsmáros, T., Kiss, H.J.M., London, G., and Nussinov, R. (2013). Structure and dynamics of molecular networks: A novel paradigm of drug discovery: A comprehensive review. *Pharmacol Ther* 138, 333-408.
- Cumberbatch, M., Tang, X., Beran, G., Eckersley, S., Wang, X., Ellston, R.P.A., Dearden, S., Cosulich, S., Smith, P.D., Behrens, C., et al. (2014). Identification of a subset of human non-small cell lung cancer patients with high PI3K $\beta$  and low PTEN expression, more prevalent in squamous cell carcinoma. *Clin Cancer Res* 20, 595-603.
- Cvetkovska, M., Rampitsch, C., Bykova, N., and Xing, T. (2005). Genomic analysis of MAP kinase cascades in *Arabidopsis* defense responses. *Plant Mol Biol Rep* 23, 331-343.
- Dardick, C., Chen, J., Richter, T., Ouyang, S., and Ronald, P. (2007). The rice kinase database. A phylogenomic database for the rice kinome. *Plant Physiol* 143, 579-586.

- Deming, D.A., Leystra, A.A., Nettekoven, L., Sievers, C., Miller, D., Middlebrooks, M., Clipson, L., Albrecht, D., Bacher, J., Washington, M.K., et al. (2014). *PIK3CA* and *APC* mutations are synergistic in the development of intestinal cancers. *Oncogene* 33, 2245-2254.
- Dinkova, T.D., De La Cruz, H.R., García-Flores, C., Aguilar, R., Jiménez-García, L.F., and De Jiménez, E.S. (2007). Dissecting the TOR–S6K signal transduction pathway in maize seedlings: Relevance on cell growth regulation. *Physiol Plant* 130, 1-10.
- Du, D., Lee, C.F., and Li, X.-Q. (2012). Systematic differences in signal emitting and receiving revealed by PageRank analysis of a human protein interactome. *PLoS ONE* 7, e44872.
- Edouard, T., Combier, J.-P., Nédélec, A., Bel-Vialar, S., Métrich, M., Conte-Auriol, F., Lyonnet, S., Parfait, B., Tauber, M., Salles, J.-P., et al. (2010). Functional effects of *PTPN11* (SHP2) mutations causing LEOPARD syndrome on epidermal growth factor-induced phosphoinositide 3-kinase/AKT/glycogen synthase kinase 3 $\beta$  signaling. *Mol Cell Biol* 30, 2498-2507.
- Fan, T., Gao, Y., Al-Shammari, A., Wang, X.J., and Xing, T. (2009). Yeast two-hybrid screening of MAP kinase cascade identifies cytosolic glutamine synthetase 1b as a tMEK2 interactive protein in wheat. *Can J Plant Pathol* 31, 407-414.
- Fang, L., Li, H., Wang, L., Hu, J., Jin, T., Wang, J., and Yang, B.B. (2014). MicroRNA-17-5p promotes chemotherapeutic drug resistance and tumour metastasis of colorectal cancer by repressing PTEN expression. *Oncotarget* 5, 2974-2987.
- Follo, M.Y., Mongiorgi, S., Bosi, C., Cappellini, A., Finelli, C., Chiarini, F., Papa, V., Libra, M., Martinelli, G., Cocco, L., et al. (2007). The Akt/mammalian target of rapamycin signal transduction pathway is activated in high-risk myelodysplastic syndromes and influences cell survival and proliferation. *Cancer Res* 67, 4287-4294.
- Foster, R., Griffin, S., Grooby, S., Feltell, R., Christopherson, C., Chang, M., Sninsky, J., Kwok, S., and Torrance, C. (2012). Multiple metabolic alterations exist in mutant PI3K cancers, but only glucose is essential as a nutrient source. *PLoS ONE* 7, e45061.
- Fuentes, E.N., Björnsson, B.T., Valdés, J.A., Einarsdóttir, I.E., Lorca, B., Alvarez, M., and Molina, A. (2011). IGF-I/PI3K/Akt and IGF-I/MAPK/ERK pathways in vivo in skeletal muscle are regulated by nutrition and contribute to somatic growth in the fine flounder. *Am J Physiol Regul Integr Comp Physiol* 300, R1532-R1542.
- García Flores, C., Aguilar, R., Reyes de la Cruz, H., Albores, M., and Sánchez de Jiménez, E. (2001). A maize insulin-like growth factor signals to a transduction pathway that regulates protein synthesis in maize. *Biochem J* 358, 95-100.
- Geng, L., Sun, B., Gao, B., Wang, Z., Quan, C., Wei, F., and Fang, X.D. (2014). MicroRNA-103 promotes colorectal cancer by targeting tumor suppressor DICER and PTEN. *Int J Mol Sci* 15, 8458-8472.
- Grabiner, B.C., Nardi, V., Birsoy, K., Possemato, R., Shen, K., Sinha, S., Jordan, A., Beck, A.H., and Sabatini, D.M. (2014). A diverse array of cancer-associated MTOR mutations are hyperactivating and can predict rapamycin sensitivity. *Cancer Discov* 4, 554-563.
- Hamel, L.P., Nicole, M.C., Duplessis, S., and Ellis, B.E. (2012). Mitogen-activated protein kinase signaling in plant-interacting fungi: Distinct messages from conserved messengers. *Plant Cell* 24, 1327-1351.
- Han, X.J., Yang, Z.J., Jiang, L.P., Wei, Y.F., Liao, M.F., Qian, Y., Li, Y., Huang, X., Wang, J.B., Xin, H.B., et al. (2015). Mitochondrial dynamics regulates hypoxia-induced migration and antineoplastic activity of cisplatin in breast cancer cells. *Int J Oncol* 46, 691-700.
- Herman, P.L., Ramberg, H., Baack, R.D., Markwell, J., and Osterman, J.C. (2002). Formate dehydrogenase in *Arabidopsis thaliana*: Overexpression and subcellular localization in leaves. *Plant Sci* 163, 1137-1145.
- Holmgren, M., and Rosenthal, J.J. (2015). Regulation of ion channel and transporter function through RNA editing. *Curr Issues Mol Biol* 17, 23-36.
- Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M., and Sakaki, Y. (2001). A comprehensive two-hybrid analysis to explore the yeast protein interactome. *Proc Natl Acad Sci USA* 98, 4569-4574.
- Iván, G., and Grolmusz, V. (2011). When the Web meets the cell: Using personalized PageRank for analyzing protein interaction networks. *Bioinformatics* 27, 405-407.
- Jelovac, D., Beaver, J.A., Balukrishna, S., Wong, H.Y., Toro, P.V., Cimino-Mathews, A., Argani, P., Stearns, V., Jacobs, L., VanDenBerg, D., et al. (2014). A *PIK3CA* mutation detected in plasma from a patient with synchronous primary breast and lung cancers. *Hum Pathol* 45, 880-883.
- Joshi, R.S., Mishra, M., Tamhane, V.A., Ghosh, A., Sonavane, U., Suresh, C.G., Joshi, R., Gupta, V.S., and Giri, A.P. (2014). The remarkable efficiency of a Pin-II proteinase inhibitor sans two conserved disulfide bonds is due to enhanced flexibility and hydrogen bond density in the reactive site loop. *J Biomol Struct Dyn* 32, 13-26.
- Kaake, R.M., Wang, X., and Huang, L. (2010). Profiling of protein interaction networks of protein complexes using affinity purification and quantitative mass spectrometry. *Mol Cell Proteomics* 9, 1650-1665.
- Keppler-Noreuil, K.M., Rios, J.J., Parker, V.E.R., Semple, R.K., Lindhurst, M.J., Sapp, J.C., Alomari, A., Ezaki, M., Dobyms, W., and Biesecker, L.G. (2015). *PIK3CA*-related overgrowth spectrum (PROS): Diagnostic and testing eligibility criteria, differential diagnosis, and evaluation. *Am J Med Genet A* 167A, 287-295.
- Keppler-Noreuil, K.M., Sapp, J.C., Lindhurst, M.J., Parker, V.E.R., Blumhorst, C., Darling, T., Tosi, L.L., Huson, S.M., Whitehouse, R.W., Jakkula, E., et al. (2014). Clinical delineation and natural history of the *PIK3CA*-related overgrowth spectrum. *Am J Med Genet A* 164, 1713-1733.
- Kihiczak, G.G., Meine, J.G., Schwartz, R.A., and Janniger, C.K. (2006). Klippel-Trenaunay syndrome: A multisystem disorder possibly resulting from a pathogenic gene for vascular and tissue overgrowth. *Int J Dermatol* 45, 883-890.
- Kim, G., Ouzounova, M., Quraishi, A.A., Davis, A., Tawakkol, N., Clouthier, S.G., Malik, F., Paulson, A.K., D'Angelo, R.C., Korkaya, S., et al. (2014). SOCS3-mediated regulation of inflammatory cytokines in PTEN and p53 inactivated triple negative breast cancer model. *Oncogene* 34, 671-680.

- Kim, M.S., Jeong, E.G., Yoo, N.J., and Lee, S.H. (2008). Mutational analysis of oncogenic AKT E17K mutation in common solid cancers and acute leukaemias. *Br J Cancer* **98**, 1533-1535.
- Kimura, N., Shimada, N., Ishijima, Y., Fukuda, M., Takagi, Y., and Ishikawa, N. (2003). Nucleoside diphosphate kinases in mammalian signal transduction systems: Recent development and perspective. *J Bioenerg Biomembr* **35**, 41-47.
- Klein, S., Stroberg, A., Ghahremani, S., and Martinez-Agosto, J.A. (2012). Phenotypic progression of skeletal anomalies in CLOVES syndrome. *Am J Med Genet A* **158A**, 1690-1695.
- Klöckener, T., Hess, S., Belgardt, B.F., Paeger, L., Verhagen, L.A.W., Husch, A., Sohn, J.-W., Hampel, B., Dhillon, H., Zigman, J.M., et al. (2011). High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. *Nat Neurosci* **14**, 911-918.
- Krallinger, M., Vazquez, M., Leitner, F., Salgado, D., Chatr-aryamontri, A., Winter, A., Perfetto, L., Briganti, L., Licata, L., Iannuccelli, M., et al. (2011). The Protein-Protein Interaction tasks of BioCreative III: Classification/ranking of articles and linking bio-ontology concepts to full text. *BMC Bioinform* **12**, S3.
- Krohn, A., Freudenthaler, F., Harasimowicz, S., Kluth, M., Fuchs, S., Burkhardt, L., Stahl, P., Tsourlakis, M.C., Bauer, M., Tennstedt, P., et al. (2014). Heterogeneity and chronology of PTEN deletion and ERG fusion in prostate cancer. *Mod Pathol* **27**, 1612-1620.
- Kurek, K.C., Luks, V.L., Ayturk, U.M., Alomari, A.I., Fishman, S.J., Spencer, S.A., Mulliken, J.B., Bowen, M.E., Yamamoto, G.L., Kozakewich, H.P.W., et al. (2012). Somatic mosaic activating mutations in *PIK3CA* cause CLOVES syndrome. *Am J Hum Genet* **90**, 1108-1115.
- Lambertucci, A.C., Lambertucci, R.H., Hirabara, S.M., Curi, R., Moriscot, A.S., Alba-Loureiro, T.C., Guimarães-Ferreira, L., Levada-Pires, A.C., Vasconcelos, D.A.A., Sellitti, D.F., et al. (2012). Glutamine supplementation stimulates protein-synthetic and inhibits protein-degradative signaling pathways in skeletal muscle of diabetic rats. *PLoS ONE* **7**, e50390.
- Li, X.-Q. (2009). Developmental and environmental variation in genomes. *Heredity* **102**, 323-329.
- Li, X.-Q. (2014). Comparative analysis of the base compositions of the pre-mRNA 3' cleaved-off region and the mRNA 3' untranslated region relative to the genomic base composition in animals and plants. *PLoS ONE* **9**, e99928.
- Li, X.-Q. (2016). Natural attributes and agricultural implications of somatic genome variation. *Curr Issues Mol Biol* **20**, 29-46.
- Li, X.-Q., and Du, D. (2014). Motif types, motif locations and base composition patterns around the RNA polyadenylation site in microorganisms, plants and animals. *BMC Evol Biol* **14**, 162.
- Li, X.-Q., Zhang, T., and Donnelly, D. (2011). Selective loss of cysteine residues and disulphide bonds in a potato proteinase inhibitor II family. *PLoS ONE* **6**, e18615.
- Lindhurst, M.J., Sapp, J.C., Teer, J.K., Johnston, J.J., Finn, E.M., Peters, K., Turner, J., Cannons, J.L., Bick, D., Blakemore, L., et al. (2011). A mosaic activating mutation in *AKT1* associated with the Proteus syndrome. *New Engl J Med* **365**, 611-619.
- Lindhurst, M.J., Wang, J., Bloomhardt, H.M., Witkowski, A.M., Singh, L.N., Bick, D.P., Gambello, M.J., Powell, C.M., Lee, C.-C.R., Darling, T.N., et al. (2014). *AKT1* gene mutation levels are correlated with the type of dermatologic lesions in patients with Proteus syndrome. *J Invest Dermatol* **134**, 543-546.
- Liu, W., Li, D., Wang, J., Xie, H., Zhu, Y., and He, F. (2009). Proteome-wide prediction of signal flow direction in protein interaction networks based on interacting domains. *Mol Cell Proteomics* **8**, 2063-2070.
- Luks, V.L., Kamitaki, N., Vivero, M.P., Uller, W., Rab, R., Bovee, J.V.M.G., Rialon, K.L., Guevara, C.J., Alomari, A.I., Greene, A.K., et al. (2015). Lymphatic and other vascular malformative/overgrowth disorders are caused by somatic mutations in *PIK3CA*. *J Pediatr* **166**, 1048-1054.
- Ma, F., Zhang, J., Zhong, L., Wang, L., Liu, Y., Wang, Y., Peng, L., and Guo, B. (2014a). Upregulated microRNA-301a in breast cancer promotes tumor metastasis by targeting PTEN and activating Wnt/ $\beta$ -catenin signaling. *Gene* **535**, 191-197.
- Ma, J., Liu, J., Wang, Z., Gu, X., Fan, Y., Zhang, W., Xu, L., Zhang, J., and Cai, D. (2014b). NF-kappaB-dependent MicroRNA-425 upregulation promotes gastric cancer cell growth by targeting PTEN upon IL-1 $\beta$  induction. *Mol Cancer* **13**, 40.
- MAPK Group (2002). Mitogen-activated protein kinase cascades in plants: A new nomenclature. *Trends Plant Sci* **7**, 301-308.
- Marsh, D.J., Trahair, T.N., and Kirk, E.P. (2011). Mutant *AKT1* in Proteus syndrome. *New Engl J Med* **365**, 2141-2142.
- Mendoza, M.C., Er, E.E., and Blenis, J. (2011). The Ras-ERK and PI3K-mTOR pathways: Cross-talk and compensation. *Trends Biochem Sci* **36**, 320-328.
- Mishra, M., Mahajan, N., Tamhane, V.A., Kulkarni, M.J., Baldwin, I.T., Gupta, V.S., and Giri, A.P. (2012). Stress inducible proteinase inhibitor diversity in *Capsicum annuum*. *BMC Plant Biol* **12**, 217.
- Modi, H., Cornu, M., and Thorens, B. (2014). Glutamine stimulates biosynthesis and secretion of insulin-like growth factor 2 (IGF2), an autocrine regulator of beta cell mass and function. *J Biol Chem* **289**, 31972-31982.
- Monet, M., Gkika, D., Lehen'kyi, V., Pourtier, A., Vanden Abeele, F., Bidaux, G., Juvin, V., Rassendren, F., Humez, S., and Prevarsakaya, N. (2009). Lysophospholipids stimulate prostate cancer cell migration via TRPV2 channel activation. *Biochim Biophys Acta Mol Cell Res* **1793**, 528-539.
- Nakayama, Y., Kusuda, S., Nagata, N., and Yamaguchi, K. (2009). Excision of a large abdominal wall lipoma improved bowel passage in a Proteus syndrome patient. *World J Gastroenterol* **15**, 3312-3314.
- Naramura, M., Nadeau, S., Mohapatra, B., Ahmad, G., Mukhopadhyay, C., Sattler, M., Raja, S.M., Natarajan, A., Band, V., and Band, H. (2011). Mutant Cbl proteins as oncogenic drivers in myeloproliferative disorders. *Oncotarget* **2**, 245-250.

- Ovečka, M., Takáč, T., Komis, G., Vadovič, P., Bekešová, S., Doskočilová, A., Smékalová, V., Luptovčiak, I., Šamajová, O., Schweighofer, A., et al. (2014). Salt-induced subcellular kinase relocation and seedling susceptibility caused by overexpression of *Medicago* SIMKK in *Arabidopsis*. *J Exp Bot* **65**, 2335-2350.
- Panetti, T.S., and Mosher, D.F. (2000). Lysophospholipid-induced cell migration. *Ann New York Acad Sci* **905**, 326-329.
- Peck, S.C. (2006). Analysis of protein phosphorylation: Methods and strategies for studying kinases and substrates. *Plant J* **45**, 512-522.
- Polden, J., McManus, C.A., Dos Remedios, C., and Dunn, M.J. (2011). A 2-D gel reference map of the basic human heart proteome. *Proteomics* **11**, 3582-3586.
- Pradella, L.M., Evangelisti, C., Ligorio, C., Ceccarelli, C., Neri, I., Zuntini, R., Amato, L.B., Ferrari, S., Martelli, A.M., Gasparre, G., et al. (2014). A novel deleterious *PTEN* mutation in a patient with early-onset bilateral breast cancer. *BMC Cancer* **14**, 70.
- Ribeiro, D.L., Pinto, M.E., Rafacho, A., Bosqueiro, J.R., Maeda, S.Y., Anselmo-Franci, J.A., Taboga, S.R., and Góes, R.M. (2012). High-fat diet obesity associated with insulin resistance increases cell proliferation, estrogen receptor, and PI3K proteins in rat ventral prostate. *J Androl* **33**, 854-865.
- Sajan, M.P., Standaert, M.L., Nimal, S., Varanasi, U., Pastoor, T., Mastorides, S., Braun, U., Leitges, M., and Farese, R.V. (2009). The critical role of atypical protein kinase C in activating hepatic SREBP-1c and NFκB in obesity. *J Lipid Res* **50**, 1133-1145.
- Schaller, G.E., and Kieber, J.J. (2002). Ethylene. In *The Arabidopsis Book*, vol. 1 (Rockville, MD, USA: American Society of Plant Biologists), p. e0071.
- Schwikowski, B., Uetz, P., and Fields, S. (2000). A network of protein-protein interactions in yeast. *Nat Biotechnol* **18**, 1257-1261.
- Shan, N., Shen, L., Wang, J., He, D., and Duan, C. (2015). MiR-153 inhibits migration and invasion of human non-small-cell lung cancer by targeting ADAM19. *Biochem Biophys Res Commun* **456**, 385-391.
- Shen, Y., Zeng, L., Zhu, A., Blanc, T., Patel, D., Pennello, A., Bari, A., Ng, S., Persaud, K., Kang, Y.K., et al. (2013). Removal of a C-terminal serine residue proximal to the inter-chain disulfide bond of a human IgG1 lambda light chain mediates enhanced antibody stability and antibody dependent cell-mediated cytotoxicity. *mAbs* **5**, 418-431.
- Singh, R., Xu, J., and Berger, B. (2008). Global alignment of multiple protein interaction networks with application to functional orthology detection. *Proc Natl Acad Sci USA* **105**, 12763-12768.
- Singh, S.S., Chauhan, A., Brockerhoff, H., and Chauhan, V.P.S. (1993). Activation of protein kinase C by phosphatidylinositol 3,4,5,-trisphosphate. *Biochem Biophys Res Commun* **195**, 104-112.
- Stulemeijer, I.J.E., Stratmann, J.W., and Joosten, M.H.A.J. (2007). Tomato mitogen-activated protein kinases LeMPK1, LeMPK2, and LeMPK3 are activated during the Cf-4/Avr4-induced hypersensitive response and have distinct phosphorylation specificities. *Plant Physiol* **144**, 1481-1494.
- Sun, S., Wen, Q., Dai, J., Xing, T., and Wang, X. (2012). Responses of *Sinningia speciosa* overexpressing *tMEK2* to temperature stresses. *Acta Hort Sin* **39**, 375-380.
- Taghnia, A.H., Mulliken, J.B., and Rogers, G.F. (2007). A case of Proteus syndrome with lateral embryonal vein and frontal intraosseous lipoma. *Cleft Palate-Craniofac J* **44**, 567-571.
- Tena, G., Boudsocq, M., and Sheen, J. (2011). Protein kinase signaling networks in plant innate immunity. *Curr Opin Plant Biol* **14**, 519-529.
- Thurston, G., Regan, S., Rampitsch, C., and Xing, T. (2005). Proteomic and phosphoproteomic approaches to understand plant-pathogen interactions. *Physiol Mol Plant Pathol* **66**, 3-11.
- Tompa, P., Davey, N.E., Gibson, T.J., and Babu, M.M. (2014). A million peptide motifs for the molecular biologist. *Mol Cell* **55**, 161-169.
- Troca-Marín, J.A., Casañas, J.J., Benito, I., and Montesinos, M.L. (2014). The Akt-mTOR pathway in Down's syndrome: The potential use of rapamycin/rapalogs for treating cognitive deficits. *CNS Neurol Disord Drug Targets* **13**, 34-40.
- Vidal, M., Brachmann, R.K., Fattaey, A., Harlow, E., and Boeke, J.D. (1996). Reverse two-hybrid and one-hybrid systems to detect dissociation of protein-protein and DNA-protein interactions. *Proc Natl Acad Sci USA* **93**, 10315-10320.
- Wang, Y., Tang, Q., Li, M., Jiang, S., and Wang, X. (2014). MicroRNA-375 inhibits colorectal cancer growth by targeting PIK3CA. *Biochem Biophys Res Commun* **444**, 199-204.
- Weston, J., Elisseeff, A., Zhou, D., Leslie, C.S., and Noble, W.S. (2004). Protein ranking: From local to global structure in the protein similarity network. *Proc Natl Acad Sci USA* **101**, 6559-6563.
- Weston, J., Kuang, R., Leslie, C., and Noble, W.S. (2006). Protein ranking by semi-supervised network propagation. *BMC Bioinform* **7**, S10.
- Winter, J.N., Jefferson, L.S., and Kimball, S.R. (2011). ERK and Akt signaling pathways function through parallel mechanisms to promote mTORC1 signaling. *Am J Physiol Cell Physiol* **300**, C1172-C1180.
- Xavier-Filho, J., Oliveira, A.E.A., da Silva, L.B., Azevedo, C.R., Venâncio, T.M., Machado, O.L.T., Oliva, M.L., Fernandes, K.V.S., and Xavier-Neto, J. (2003). Plant insulin or glucokinase: A conflicting issue. *Braz J Plant Physiol* **15**, 67-78.
- Xiao, G.H., Jin, F., and Yeung, R.S. (1995). Germ-line *Tsc2* mutation in a dominantly inherited cancer model defines a novel family of rat intracisternal-A particle elements. *Oncogene* **11**, 81-87.
- Xing, T., and Laroche, A. (2011). Revealing plant defense signaling: Getting more sophisticated with phosphoproteomics. *Plant Signal Behav* **6**, 1469-1474.
- Xing, T., Ouellet, T., and Miki, B.L. (2002). Towards genomic and proteomic studies of protein phosphorylation in plant-pathogen interactions. *Trends Plant Sci* **7**, 224-230.
- Xing, T., Rampitsch, C., Miki, B.L., Mauthe, W., Stebbing, J.-A., Malik, K., and Jordan, M. (2003). MALDI-Qq-TOF-MS and transient gene expression analysis indicated co-enhancement of β-1,3-glucanase and endochitinase by



- tMEK2 and the involvement of divergent pathways. *Physiol Mol Plant Pathol* 62, 209-217.
- Xing, T., Rampitsch, C., Sun, S., Romanowski, A., Conroy, C., Stebbing, J.-A., and Wang, X. (2008). TAB2, a nucleoside diphosphate protein kinase, is a component of the tMEK2 disease resistance pathway in tomato. *Physiol Mol Plant Pathol* 73, 33-39.
- Yang, Q., Shao, Y., Shi, J., Qu, Y., Wu, K., Dang, S., Shi, B., and Hou, P. (2014a). Concomitant *PIK3CA* amplification and *RASSF1A* or *PAX6* hypermethylation predict worse survival in gastric cancer. *Clin Biochem* 47, 111-116.
- Yang, T.-S., Yang, X.-H., Chen, X., Wang, X.-D., Hua, J., Zhou, D.-L., Zhou, B., and Song, Z.-S. (2014b). MicroRNA-106b in cancer-associated fibroblasts from gastric cancer promotes cell migration and invasion by targeting PTEN. *FEBS Lett* 588, 2162-2169.
- Ye, Z.-B., Ma, G., Zhao, Y.-H., Xiao, Y., Zhan, Y., Jing, C., Gao, K., Liu, Z.-H., and Yu, S.-J. (2015). miR-429 inhibits migration and invasion of breast cancer cells in vitro. *Int J Oncol* 46, 531-538.
- Yu, M., Trobridge, P., Wang, Y., Kanngurn, S., Morris, S.M., Knoblauch, S., and Grady, W.M. (2014). Inactivation of TGF- $\beta$  signaling and loss of *PTEN* cooperate to induce colon cancer *in vivo*. *Oncogene* 33, 1538-1547.
- Zardavas, D., Phillips, W.A., and Loi, S. (2014). *PIK3CA* mutations in breast cancer: Reconciling findings from preclinical and clinical data. *Breast Cancer Res* 16, 201.
- Zhang, X., Perica, T., and Teichmann, S.A. (2013). Evolution of protein structures and interactions from the perspective of residue contact networks. *Curr Opin Struct Biol* 23, 954-963.
- Zhu, J., Chen, L., Zou, L., Yang, P., Wu, R., Mao, Y., Zhou, H., Li, R., Wang, K., Wang, W., et al. (2014). MiR-20b, -21, and -130b inhibit PTEN expression resulting in B7-H1 over-expression in advanced colorectal cancer. *Hum Immunol* 75, 348-353.



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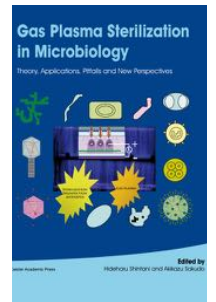
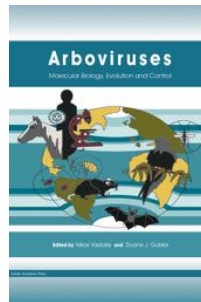
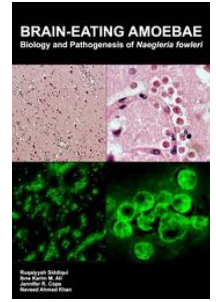
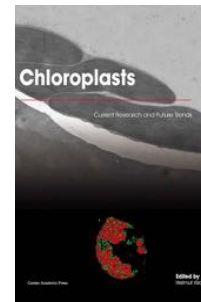
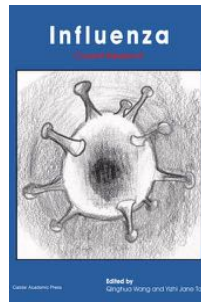
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