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Inositol-1,4,5-trisphosphate 3-kinase-A (ITPKA) is frequently over-expressed and functions as an oncogene in several tumor types

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Abstract

At present targeted tumor therapy is based on inhibition of proteins or protein mutants that are up-regulated in tumor but not in corresponding normal cells. The actin bundling Inositol-trisphosphate 3-kinase A (ITPKA) belongs to such molecular targets. ITPKA is expressed in a broad range of tumor types but shows limited expression in normal cells. In lung and breast cancer expression of ITPKA is stimulated by gene body methylation which increases with increasing malignancy of these tumors but is not detectable in the corresponding normal tissues. Since ITPKA gene body methylation occurs early in tumor development, it could serve as biomarker for early detection of lung cancer. Detailed mechanistic studies revealed that down-regulation of ITPKA in lung adenocarcinoma cancers reduced both, tumor growth and metastasis. It is assumed that tumor growth is stimulated by the InsP₃Kinase activity of ITPKA and metastasis by its actin bundling activity. A selective inhibitor against the InsP₃Kinase activity of ITPKA has been identified but compounds inhibiting the actin bundling activity are not available yet. Since no curative therapy option for metastatic lung or breast tumors exist, therapies that block activities of ITPKA may offer new options for patients with these tumors. Thus, efforts should be made to develop clinical drugs that selectively target InsP₃Kinase activity as well as actin bundling activity of ITPKA.

Keywords

ITPKA; Lung cancer; Small molecule inhibitors; Inositol; Actin

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Conflict of interest

The authors declare no conflict of interest.

1. Introduction

In the past phenotype based screens were used to identify drugs that target certain tumor types such as ovarian cancer. At present the pharmacology industry has switched almost entirely to target based screens, using molecular targets such as mutant epidermal growth factor (EGFR) or down-stream effectors of EGFR, like phosphoinositide 3-kinase (PI3K) [1,2]. The PI3K family and the Inositoltrisphosphate 3-kinase (ITPK) family are related because both phosphorylate inositol phosphates. However, although the isoform A of ITPK has been identified as oncogene, much less is known about the role of ITPKA in cancer progression as compared to PI3K. This review gives an overview about the modest information currently available about the physiological and the oncogenic role of ITPKA, with the goal of stimulating research and knowledge in this field.

2. Discovery and catalytic role of ITPKs

In addition to PI3K-mediated phosphorylation of the membrane bound lipid phosphatidylinositol biphosphate (PIP₂) to phosphatidylinositol 3,4,5 trisphosphate (PIP₃), PIP₂ can be hydrolyzed by phospholipase C to the membrane bound diacylglycerol and to soluble cytosolic inositol trisphosphate (Ins(1,4,5)P₃). Ins(1,4,5)P₃ binds to the endoplasmatic (ER) Ins(1,4,5)P₃ receptor, leading to calcium release from the ER. This Ins(1,4,5)P₃ signal can be terminated by two different enzymes; Inositol-trisphosphate 3-kinases (InsP₃Kinase; gene name: ITPK) and an Inositoltrisphosphate 5' phosphatases (gene name: INPP5A) (Fig. 1A). The product of InsP₃Kinase Ins(1,3,4,5)P₄ has second messenger functions [3,4] and is rapidly phosphorylated by different InsPKinases (ITPK) to higher phosphorylated inositols [5]. Research on InsP₃Kinases started in the 1980s. InsP₃Kinase-activity was first measured in *xenopus* oocytes, rat liver, pancreas and brain [6]. In addition, Steward et al. (1986) [7] detected InsP₃Kinase activity in Jurkat T-cells. In 1991 Takazawa et al. [8] were able to clone the first InsP₃Kinase, which therefore was named InsP₃Kinase-A (gene name: ITPKA). Thereafter, two further InsP₃Kinase isoenzymes were cloned; InsP₃Kinase-B and InsP₃Kinase-C (ITPKB and ITPKC) [9–11]. The catalytic domains of these isoenzymes are highly homologous, but the N-termini show large differences in size and function. The N-termini of ITPKA and ITPKB include an actin binding domain, mediating localization to F-actin [12,13]. The N-terminus of ITPKB additionally includes a nuclear localization signal, and thus the enzyme shuttles between the cytosol and nucleus [14]. The latter is also true for ITPKC [15]. In addition to the different cellular localization, expression also differs between the isoforms. Northern blot analysis revealed ubiquitous expression of ITPKB while expression of ITPKA was only detected in brain and testis [16]. The genes of ITPKA and ITPKB are located at 15q15.1 or 1q42.12, respectively (<http://www.genecards.org>).

The physiological roles of the isoforms were mainly studied by the use of knock-out mice. ITPKA knock-out mice exhibit increased synaptic plasticity and slight impairments of learning and memory [17,18], while deletion of ITPKB resulted in impaired stem cell homeostasis of immune cells [19]. ITPKC knock-out mice do not show an obvious altered phenotype [20] but a clinical relevant mutation of ITPKC is described in Kawasaki disease [21]. It is suggested that in T-cells ITPKC is a negative regulator, therefore Kawasaki

disease-associated down-regulation of ITPKC results in over activation of T-cells [22]. In summary the ITPK proteins have distinct cellular functions because of their different cellular localization and tissue expression.

Among the ITPK-isoforms ITPKA is the most specialized one. In cells it is exclusively bound to F-actin resulting in cross-linking of actin filaments [12,23]. Thus, based on this function and on its InsP₃Kinase activity, ITPKA has two very distinct functions, regulating both, calcium signaling and actin dynamics.

3. Physiological role of ITPKA

The physiological role of ITPKA is based on its bi-functionality; it regulates actin dynamics as well as Ins(1,4,5)P₃-mediated calcium signals. Actin is found in almost all eukaryotic cells in two forms: filamentous F-actin consists of two intertwined strands, that drives many cellular processes including cell motility and muscle contraction, and the monomer from which it is produced, globular or G-actin (reviewed in [24]). ITPKA regulates actin dynamics by binding with its homodimeric N-terminal actin binding domain (ABD) to F-actin. The bulky C-terminus, which includes the InsP₃Kinase-domain, acts as spacer between actin filaments resulting in formation of loose networks of F-actin bundles (Fig. 1B; [23]).

Calcium is an ubiquitous second messenger that is involved in many signal transduction pathways, including protein kinase C and CAMKII signaling (reviewed in [25,26]). Cellular calcium signals are regulated by the InsP₃Kinase activity of ITPKA. It phosphorylates the calcium-mobilizing second messenger Ins(1,4,5)P₃ at 3' position, thereby producing Ins(1,3,4,5)P₄. Since the Ins(1,4,5)P₃ loop binds Ins(1,4,5)P₃ with high affinity (K_m 0.4 μM, [27]), but no other InsP-isomers or phosphatidylinositol phosphates, ITPKA is a highly specialized enzyme [28]. Ins(1,3,4,5)P₄ is substantially involved in the control of Ins(1,4,5)P₃-mediated calcium release. The (1,4,5)P₃ phosphatase INPP5A binds Ins(1,3,4,5)P₄ with tenfold higher affinity than Ins(1,4,5)P₃, resulting in decreased (1,4,5)P₃ dephosphorylation [29]. Therefore, production of Ins(1,3,4,5)P₄ increases half-life of Ins(1,4,5)P₃, thus Ins(1,4,5)P₃-mediated calcium release from the endoplasmic reticulum. Based on this property, in the absence of ITPKA calcium release is shortened and calcium-induced calcium entry abrogated [29,30].

The *itpka* gene is most highly expressed in the brain, with highest expression in pyramidal neurons of the CA1 region and the dentate gyrus of the hippocampus. In addition, high levels of ITPKA were detected in pyramidal neurons of the neocortex and in Purkinje cells of the cerebellum [31,32]. Furthermore, Vanweyenberg et al. (1996) [16] reported that mRNA of *itpka* is also expressed in testis. In neurons, ITPKA is concentrated at postsynaptic densities (PSD) of hippocampal dendritic spines [33]. Dendritic spines are neuronal protrusions, each of which receives input typically from one excitatory synapse. They contain neurotransmitter receptors, organelles, and signaling systems essential for synaptic function and plasticity. Numerous brain disorders are associated with abnormal dendritic spines (reviewed in [34,35]). At PSDs scaffold proteins, F-actin and receptors (NMDAR, AMPAR etc.) are highly concentrated to allow an efficient signal transmission from the pre-

to the post-synapsis. During learning and memory processes formation, maintenance and morphology of dendritic spines is highly plastic (“synaptic plasticity”). Efficiency of signal transmission depends on the density of PSD receptors (in particular AMPAR) and on the size of dendritic spines. Whereas translocation of AMPAR to the postsynaptic membrane is mainly controlled by calcium signals, the size of dendritic spines is regulated by actin binding proteins (reviewed in [34,35]). ITPKA is involved in both, the control of calcium signals and the control of dendritic spine morphology [33,18,36,37]. Therefore, ITPKA depletion in mice results in altered synaptic plasticity and thus in impaired learning and memory [17,18].

4. ITPKA expression in lung and other cancers

Normally, expression of neuronal genes is suppressed in most non-neuronal tissue. However, many tumor cells overexpress the genes. Therefore, different tumor entities are able to express ITPKA, including pancreas, testis, thyroid, breast, lung, colon, liver, prostate, uterus and skin [38]). Wang et al. (2016) [39] revealed that ITPKA belongs to the top 20 up-regulated genes in lung adenocarcinomas but was not expressed in matched non-malignant lung tissue. Furthermore, lung cancer cell lines expressed significantly higher levels than immortalized respiratory epithelial cells. In addition to non-small cell lung cancers and cell lines (squamous cell and adenocarcinomas) expression of ITPKA was elevated in small cell lung cancer (SCLC) cell lines. Thus ITPKA was elevated in both neuroendocrine (SCLC) and non-SCLC lung cancers.

These findings led us to examine The Cancer Genome Atlas (TCGA) data set in greater detail (Fig. 2A and B, Table 1). The TCGA is a large multimodality study of the major human tumor types sponsored by the National Cancer Institute and the National Human Genome Research Institute, USA (<https://cancergenome.nih.gov>). The TCGA data are constantly being updated, and the data we analyzed were downloaded during May and June 2015. Gene expression data were from RNASeq examination, and there were no protein data available for ITPKA. Thus, all expression data from the TCGA must be regarded as preliminary. When the values of all tumor samples (“pan tumor”) were compared to the values of all non malignant tissues (“pan non malignant”) the tumor values were highly significantly greater (Fig. 2A). However, the values in both tumors and non malignant tissues were highly variable, as were the ratios between the tumors and their corresponding non malignant tissues (Fig. 2B, Table 1). For some tumor types, corresponding non tumor tissues were not available, and for these we used the pan non malignant tissues for comparison. In 19 tumor types ITPKA values were significantly higher in tumors (Table 1, marked in red) in seven types there were no significant differences (Table 1, marked in green), and in five the tumor values were lower than in the corresponding non malignant or in the pan non malignant tissues (Table 1, marked in yellow). The latter figure drops to four types if the two melanoma groups (cutaneous and uveal) are combined. Three examples of each of these comparison types are illustrated in Fig. 2B. Of interest, the values in brain (presumably mainly or entirely from neurons) is significantly higher than in glial derived tumors. We noted that very high levels of ITPKA were expressed in non malignant colorectal and gastric tissues, nearly as high as in brain tissues. The GI tract (and colon in particular) [40,41] are tissues that show considerable amounts of age related methylation.

We investigated whether methylation (and resultant over expression of ITPKA) were age related in the colorectum, but found no evidence (data not shown).

Thus, ITPKA is expressed in a broad range of tumor entities among which breast, lung and renal cell carcinomas show the highest expression ratios compared to corresponding non-malignant tissues.

5. Regulation of ITPKA expression in tumor cells

Expression of neuronal genes in non-neuronal cells is suppressed by the transcription suppressor RE1 Silencing Transcription Factor (REST-1). However, expression of this transcription factor can be down-regulated or mutated in many tumors, resulting in re-expression of neuronal genes such as glycine receptor $\alpha 1$ subunit [42–47]. In many colon cancers deletion of the REST gene has been detected and in breast and prostate cancer decreased REST expression correlates with aggressive cancer phenotypes [46]. In non-small-cell lung carcinoma (NSCLC) loss of the co-repressor complex BRM-BRG1 has been found [47] while small-cell lung carcinoma (SCLC) show expression of the dominant negative isoform REST4 [42].

A detailed promoter-analysis revealed that also expression of the *itpka* gene is regulated by REST-1 in NCI-H1299 lung cancer and T47D breast cancer cells [48]. The TATA-box less *itpka* promoter is positively controlled by Sp1 and negatively by REST-1. This negative control is abrogated by overexpression of the dominant negative isoform REST4 resulting in re-expression of ITPKA [48]. However, if this is true in general has to be shown.

To examine the mechanism by which ITPKA is selectively expressed in many tumor types, the TCGA data base for lung cancers and lung cancer cell lines were examined [39]. Only occasional mutations and low level copy gains were noted, indicating that these mechanisms could not represent important mechanisms for gene activation in tumors. To explore epigenetic methods, the structure of the gene was examined. Two CPG islands were identified, one in the promoter region, and one between exon 2 and intron 5. The CpG island in the promoter region had low levels of partial methylation, while five of six probes in the body region showed differential methylation in tumor cells. There was a positive correlation between gene body methylation and expression. These results also held true for breast cancers. When methylation positive cell lines were exposed to a DNA methyltransferase inhibitor, both methylation levels and gene expression were decreased. Knockdown of ITPKA expression by small interfering RNA oligomers suppressed cell proliferation and colony formation. A more stable knockdown experiment, using lentivirus-based short hairpin RNA was used to confirm these results. In addition, stable knockdown of ITPKA suppressed xenograft growth in immunosuppressed mice. These findings confirmed that gene body methylation was directly related to gene expression. Further experiments demonstrated that DNA methylation of the gene body was regulated by the binding of the transcription factor SP1 to the *itpka* promoter.

Interestingly, ITPKA expression seems to be induced by oncogenes: Already in 1992 Benz et al. [49] revealed that overexpression of ERBB2 in the breast cell line MCF-7 induced

ITPKA expression. Consistent with this result, 21 years later Pincini et al. (2013) [50] showed that ITPKA belongs to the invasive signature of p130Cas/ErbB2 transformed MCF10A.B2 breast cancer cells. In addition to ErbB2, also the *v-src* oncogene seems to be sufficient to induce expression of ITPKA. Woodring and Garrison (1996) [51] revealed that Rat-1 fibroblasts transformed with the *v-src* oncogene show high levels of ITPKA while expression of the isoform B was not affected.

DNA methylation in promoters is well known to silence genes and is the presumed therapeutic target of methylation inhibitors [52]. However, gene body methylation may also occur, and often is positively correlated with expression [52]. We [39] and others [52] have shown that 5-aza-2'-deoxycytidine treatment not only reactivates genes but decreases the overexpression of genes, many of which are involved in metabolic processes regulated by c-MYC. Downregulation is caused by DNA demethylation of the gene bodies and restoration of high levels of expression requires remethylation by DNMT3B. Gene body methylation may, therefore, be an unexpected therapeutic target for DNA methylation inhibitors, resulting in the normalization of gene overexpression induced during carcinogenesis. These results provide direct evidence for a causal relationship between gene body methylation and transcription. Thus, expression of ITPKA is regulated by different mechanism of which gene body methylation provides the best possibility to target expression of ITPKA in tumor cells.

6. ITPKA is an oncogene and is involved in metastasis

As previously mentioned, stable knockdown of ITPKA in high expressing tumor cells resulted in decreased cell growth, colony formation and suppression of xenograft growth in immunosuppressed mice. These findings provide powerful evidence that ITPKA functions as an oncogene, and that the malignant properties of ITPKA expressing tumors are at least partially dependent on expression of the gene.

It was found that high ITPKA expression was significantly correlated with vascular invasion and poor survival of hepatocellular carcinoma cells [53] and Wang et al. (2016) [39] revealed that ITPKA expression in lung cancers appeared at the earliest preinvasive stages and progressively increased during multistage pathogenesis. This finding that ITPKA expression is a very early event during carcinogenesis, is consistent with the concept that ITPKA plays a pivotal role in the onset of the malignant phenotype. These data as well as the finding of Pincini et al. (2013) [50] (see above) that ITPKA belongs to the invasive signature of p130Cas/ErbB2 transformed breast cancer cells show that in different tumor entities expression of ITPKA is associated with malignancy of tumor cells.

In line with these results it was shown that down-regulation of ITPKA in tumor cells with high endogenous ITPKA expression decreased, while overexpression in tumor cells with low endogenous expression increased trans-migration [30,38]. Moreover, overexpression of ITPKA in the lung adenocarcinoma cell line H1299 increased metastases 4-fold [30]. In addition stable down-regulation of ITPKA in the same cell line reduced metastasis in a SCID mouse model by 95% (SW unpublished results). These data clearly show that ITPKA plays an important role for metastasis of lung cancer and other tumor cells.

7. Mechanism and role of ITPKA in metastasis

ITPKA is a bi-functional protein; it phosphorylates $\text{Ins}(1,4,5)\text{P}_3$ by its kinase activity and cross-links F-actin by its F-actin bundling activity (Fig. 1A and B). Hence, in principle both activities could account for the ITPKA-promoting effects on lung cancer metastasis. A rescue experiment, where wt ITPKA and a mutant lacking InsP_3 Kinase activity (“kinase-dead mutant”) were re-expressed in ITPKA-depleted lung carcinoma cells, could give clues to its function in tumor cells. Transmigration experiments revealed that re-expression of wt ITPKA completely restored reduced transmigration of ITPKA-depleted cells. Interestingly, also re-expression of a kinase-dead mutant rescued reduced transmigration, but only by 60% [30]. Thus, it seems that both, the InsP_3 Kinase and the F-actin bundling activity of ITPKA mediate the metastasis-promoting effect of ITPKA.

We assume that the F-actin bundling activity of ITPKA is required for formation of different kinds of cellular protrusions which may be necessary for adhesion, transmigration and invasion [30,38]. Inhibition of the InsP_3 Kinase activity reduced proliferation and adhesion of lung cancer cells [54]. Therefore, combined inhibition of F-actin bundling and InsP_3 Kinase activity should inhibit metastasis at early (adhesion, invasion) and late steps (colonization at secondary sites) of metastasis (see Fig. 3).

8. ITPKA gene body methylation as a cancer biomarker

As previously discussed, in many organ systems ITPKA is differentially expressed in tumor cells but not in corresponding non malignant tissues. Expression begins at an early stage of tumorigenesis. As expression and gene body methylation are tightly regulated, methylation may be used as a biomarker for early tumor detection in tissues. Methylation probes that can be utilized in formalin fixed paraffin embedded materials were designed and tested [39]. Most benign cell lines and tissues lacked methylation, while lung, breast and other tumors showing differential expression of ITPKA were positive, suggesting that for many tumor types ITPKA methylation is a tissue specific marker for tumor cells in order to differentiate them from nonmalignant tissues. In addition, noninvasive techniques could be used to analyze ITPKA gene body methylation. In both, circulating DNA (ctDNA) and circulating tumor cells (CTCs) [55], the level of ITPKA methylation should correspond to the level of the primary tumor. Since ITPKA is not expressed in immune cells (own unpublished results, SW), the likelihood for detection of false positive results is very small. Thus, the development of a standardized test to detect ITPKA methylation in ctDNA and/or in CTCs would provide a powerful tool to noninvasively detect tumor cells at an early stage.

9. Targeting of ITPKA

In order to block the metastatic potential of ITPKA it is necessary to inhibit both, its actin bundling and its InsP_3 Kinase activity. By performing a high throughput screen [56] using the purified InsP_3 Kinase domain of ITPKA, we identified a small molecule inhibitor against the InsP_3 kinase activity of ITPKA. The chemical name of this nitrophenolic substance is 2-[3,5-dimethyl-1-(4-nitrophenyl)-1H-pyrazol-4-yl]-5,8-dinitro-1H-benzo[de]isoquinoline-1,3(2

H)-dione, we named it BIP-4. BIP-4 is a new compound that has been not used in clinical trials and no other targets for BIP-4 have been published [54].

BIP-4 is competitive to Ins(1,4,5)P₃ and inhibits InsP₃Kinase activity with an in vitro IC₅₀ value of 157 nM. The nitro-groups of the benzyloquinoline ring bind to the Ins(1,4,5)P₃ binding pocket and interact with the same amino acids as the phosphate group one and four of the Ins(1,4,5)P₃ molecule, resulting in replacement of Ins(1,4,5)P₃ ([54] and Fig. 4A). This property makes BIP-4 a highly selective InsP₃Kinase inhibitor, because the Ins(1,4,5)P₃-binding pocket is unique among the InsP-kinases [28]. Based on the high selectivity of BIP-4 for the Ins(1,4,5)P₃ binding pocket, BIP-4 does not block the actin bundling activity of ITPKA. Thus, in order to inhibit the actin bundling activity a new small molecule inhibitor has to be identified.

As BIP-4 possesses three polar nitro groups, cellular uptake is relatively weak (6%). Thus, after addition of 3 μM BIP-4 to the cell culture medium, the cellular concentration of BIP-4 is 180 nM, which corresponds to the in vitro IC₅₀ value to inhibit InsP₃Kinase activity. When adding 3 μM BIP-4 to the cell culture medium, both, proliferation and adhesion of lung cancer cells is inhibited while migration is not affected. Hence, inhibition of cellular InsP₃Kinase reduces important steps in the metastatic cascade (see Fig. 3).

For preclinical trials the structure of BIP-4 has to be optimized. The phenyl- and the pyrazole-group of BIP-4 (Fig. 4B) do not interact with the Ins(1,4,5)P₃ binding pocket. These residues could be deleted to produce a smaller molecule with improved membrane-permeability. In addition, the nitro groups of BIP-4 could be potentially toxic, thus efforts should be made to replace these groups, for instance by carboxyl-groups. Furthermore, it should be tested if the modified molecule passes the brain-blood barrier.

Inhibitors against the actin bundling activity of ITPKA are not yet available. Since inhibition of cellular InsP₃Kinase activity inhibits proliferation and adhesion of lung cancer cells, we assume that inhibition of the actin bundling activity of ITPKA will result in reduction of both invasion and migration. In this case both growth and dissemination of lung tumors could be blocked. Based on the fact that until now, there is no curative therapy option for metastatic lung or breast tumors, such an approach would be a great benefit for these cancer patients. Thus, it is of high interest to identify inhibitors against the actin bundling activity of ITPKA.

The actin binding domain of ITPKA has no homology to other actin binding domains, which is an advantage for specific inhibition of ITPKA's actin bundling activity. In contrast to the catalytic domain whose 3-D-structure has been identified, the structure of the ABD is unknown. The best way to identify small molecules that interact with the ABD is to crystallize the ABD and elucidate its 3-D-structure. Then, in silico screens could be performed and candidate drugs validated by in vitro assays.

10. Summary/future directions

Different groups have demonstrated that ITPKA plays a crucial role in the malignant progression of tumor cells, and its expression may be a negative prognostic marker for lung

and liver tumors. ITPKA is differentially over expressed in many tumor types suggesting that it may play a role in their pathogenesis. ITPKA is an oncogene different from most “classic” oncogenes. Classic protooncogenes like Rat sarcoma (Ras), PI3K or EGFR, Protein kinase B or Mitogen Activated Protein Kinases (MAPK) are ubiquitously expressed (<https://www.ebi.ac.uk/gxa/home>) and become oncogenes after mutations or amplifications in cancer cells leading to up-regulated signal transduction and increased cellular proliferation. ITPKA, by contrast, has a limited tissue distribution but is over expressed in many different solid tumors. In tumors not the mutated form of ITPKA has oncogenic function, but its dual actin bundling and InsP₃Kinase activity, resulting in regulation of both, actin dynamics and calcium signaling. In addition, its method of expression regulation, via gene body methylation, while not unique, is unusual for an oncogene. Furthermore, ITPKA does not belong to the protein kinases, most of which possesses the highly conserved DFG-motif in their ATP binding pocket. The unique Ins (1,4,5)P₃ binding loop inside the catalytic domain of ITPKA [28] enables selective inhibition of ITPKA’s InsP₃Kinase activity. Also the actin binding motif of ITPKA does not belong the classic actin binding motifs [12], thus offering the possibility to develop specific inhibitors against the actin bundling activity of ITPKA. Together these properties, different from classical oncogenes, make ITPKA a very promising target for specific tumor therapy. Moreover, since ITPKA is expressed very early during multistage pathogenesis of lung cancers through gene body methylation, the signal of ITPKA gene body methylation in circulating DNA or circulating tumor cells could serve as blood biomarker for early detection of lung cancer.

ITPKA is an important molecule, and plays crucial roles in both physiology and cancer. Effective therapies that block the activities of this molecule may have a major role in the therapeutic control of several common cancer types.

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Fig. 1.

Ins(1,4,5)P₃-mediated cellular signaling. (A) Ins(1,4,5)P₃ binds to the IP₃R at the ER, resulting in calcium release. This Ins(1,4,5)P₃-mediated calcium signal is terminated by two different enzymes: a phosphatase (5'PPT) which dephosphorylates Ins(1,4,5)P₃ at 5' position and a kinase (ITPK) that phosphorylates Ins(1,4,5)P₃ at 3' position to Ins(1,3,4,5)P₄. The 5'PPT binds Ins(1,3,4,5)P₄ with ten-fold higher affinity as compared to Ins(1,4,5)P₃ leading to reduced dephosphorylation of Ins(1,4,5)P₃, thus to elongated calcium release from the ER. In addition, Ins(1,3,4,5)P₄ is the substrate for formation of all higher phosphorylated inositols. PLC: Phospholipase C, 5'PPT: Phosphatase, that dephosphorylates (1,4,5)P₃ and (1,3,4,5)P₄ at 5' position, ER: endoplasmic reticulum, IP₃R: Inositol trisphosphate receptor. (B) The actin binding domains of ITPKA molecules form homodimers, resulting in bundling of actin filaments. The bulky C-terminal InsP₃Kinase-domains spreads actin filaments in a way that the bundled filaments are cross-linked to loose F-actin networks.

Fig. 2.

Database analysis of ITPKA expression in tumor and corresponding normal tissue. (A) The data consisted of 8953 tumors of 29 tumor types and 726 corresponding non malignant tumors. The values of all the tumor samples in the TCGA data base ($n = 8953$) from 29 tumor types were compared to the values of all corresponding non malignant tissues ($n = 726$) from 21 tumor types. (B) Patterns of ITPKA expression in tumors compared to corresponding non malignant tissues or pan non malignant (if corresponding tissue data were not available). Three examples of each of the three patterns are presented. The top row demonstrates examples where the values in tumors were higher, the middle row demonstrates examples where there were no significant differences, and the bottom row demonstrates examples where the values in the tumors were significantly lower. Note the

glial tumors (lower row, right panel) consist of data from lower grade gliomas (astrocytomas and oligodendrogliomas) combined with glioblastomas.

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Fig. 3.

Metastasis of tumor cells. In response to extracellular stimuli, cells from the primary tumor adhere to the extracellular matrix (1), invade the ECM (2), disseminate through the blood stream (3, 4), extravasate and form metastases at organs distant from the primary tumor (colonization at distant sites). In the blood the tumor cells are associated with platelets (red) which protect them from immune cell (blue) attack. Colonization of tumor cells at secondary sites is stimulated by tumor associated fibroblasts (green) and by tumor associated macrophages (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4.

Structure of the InsP₃kinase inhibitor BIP-4. (A) Model of BIP-4 (green) and Ins(1,4,5)P₃ (grey) interaction with amino acids of the Ins(1,4,5)P₃ binding pocket. (B) BIP-4 consists of a benzisochinoline, a pyrazole and a phenyl-group. Only the benzisochinoline group binds to the Ins(1,4,5)P₃ binding pocket. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

ITPKA expression in tumor and corresponding normal tissue. The TCGA database was analyzed for mRNA expression of ITPKA and four main groups were defined from this analysis (please also see legend of Fig. 2). In groups 1–3, paired malignant and corresponding non malignant tissues were available. For Group 4, corresponding non malignant tissue data were unavailable. We compared tumor data with “pan non malignant” tissues – i.e. combining all of the available non-malignant tissue data available in the TCGA data set.

	Cancer type	Tumor sample size	Non-malignant sample size	P-value	Median of tumor	Median of Non-malignant
Group 1 (Tumors are significantly higher)	Breast invasive carcinoma	1095	113	8.21E-49	3.31	0.16
	Lung squamous cell carcinoma	502	51	1.09E-41	5.29	1.96
	Lung adenocarcinoma	515	59	1.00E-31	6.49	2.21
	Kidney Chromophobe	66	25	4.59E-26	7.04	1.61
	Kidney renal clear cell carcinoma	533	72	4.93E-25	3.75	0.70
Group 2 (no significant difference)	Thyroid carcinoma	505	59	1.30E-16	3.58	1.93
	Liver hepatocellular carcinoma	96	50	2.62E-11	7.22	4.71
	Head and Neck squamous cell carcinoma	520	44	3.17E-10	5.07	3.38
	Kidney renal papillary cell carcinoma	290	32	9.69E-07	2.72	1.06
	Stomach adenocarcinoma	415	35	0.02	7.57	8.11
	Bladder Urothelial Carcinoma	408	19	0.06	3.90	3.20
	Esophageal carcinoma	173	11	0.31	6.51	8.23
	Cholangiocarcinoma	36	9	0.39	5.63	5.18
	Uterine Corpus Endometrial Carcinoma	176	24	0.79	3.89	3.94
	Colon adenocarcinoma	379	51	2.06E-23	7.37	9.41
Group3 (Tumors are significantly lower)	Prostate adenocarcinoma	497	52	1.14E-10	2.29	3.52
	Brain Lower Grade Glioma & Glioblastoma multiforme	516	5	1.03E-06	6.71	10.49
Group4-1 (Tumors are significantly higher than pan non-malignant)	Sarcoma	259	2	2.40E-66	6.70	3.07
	Pancreatic adenocarcinoma	178	4	4.09E-52	6.71	4.58
	Adrenocortical carcinoma	79	0	1.37E-50	8.41	NaN
	Cervical adenocarcinoma	300	3	4.42E-38	5.80	2.75
	Testicular Germ Cell Tumors	150	0	1.76E-27	5.76	NaN
	Mesothelioma	87	0	2.38E-18	5.83	NaN
	Uterine Carcinosarcoma	57	0	6.44E-09	5.51	NaN
	Acute Myeloid Leukemia	329	0	6.01E-05	4.08	NaN

	Cancer type	Tumor sample size	Non-maglinant sample size	P-value	Median of tumor	Median of Non-malignant
Group4-2 (No significant difference)	Ovarian serous cystadenocarcinoma	262	0	1.20E-04	3.80	NaN
	Pheochromocytoma and Paraganglioma	179	3	4.18E-04	3.89	6.93
	Thymoma	120	2	0.02	3.64	1.70
	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	48	0	0.22	3.50	NaN
Group4-3 (Tumors are significantly lower than pan non-malignant)	Uveal Melanoma	80	0	7.03E-04	1.87	NaN
	Skin Cutaneous Melanoma	103	1	1.76E-03	3.98	3.92
	Pancancer	8953	726	4.3033E-86	4.90	2.24