

Insulin receptor tyrosine kinase substrate in health and disease (Review)

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Abstract. Insulin receptor (IR) tyrosine kinase substrate (IRTKS) was first identified >20 years ago as a tyrosine-phosphorylated IR substrate and subsequently characterized as a protein containing an inverse-Bin-amphiphysin-Rvs domain.

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Abbreviations: A/E, attaching and effacing; BAIAP2, brain-specific angiogenesis inhibitor 1-associated protein 2; BAIAP2L1, BAIAP2-like 1; BAR, Bin-amphiphysin-Rvs; CRC, colorectal cancer; EHEC, Enterohemorrhagic *E. coli*; EGFR, epidermal growth factor receptor; Esp8, EGFR kinase substrate 8; EspFU, *E. coli*-secreted protein F-like protein encoded on prophage U; ERK, extracellular regulated protein kinase; F-actin, fibrous actin; F-BARs, Fes/CIP4 homology-BAR; FGFR, fibroblast growth factor receptor; FMNL2, formin-like 2; GBD, GTPase binding domain; GC, gastric cancer; GSK-3 β , glycogen synthase kinase-3 β ; hAMSC, human amniotic mesenchymal stromal cell; HCC, hepatocellular carcinoma; H3K9me3, histone H3 lysine 9 trimethylation; HP1 α , heterochromatin protein 1- α ; I-BARs, inverse-BAR; IFN, interferon; IMD, IRSp53/MIM homology domain; IR, insulin receptor; IRSp53, IR tyrosine kinase substrate p53; IRTKS, IR tyrosine kinase substrate; MAVS, mitochondrial antiviral signaling protein; MDA5, melanoma differentiation-associated protein 5; MIM, missing-in-metastasis; mTOR, mammalian target of rapamycin; N-WASP, neuronal WASP; OC, ovarian cancer; PC, pancreatic cancer; PCBP2, poly(rC) binding protein 2; PI3K, phosphatidylinositol 3-kinase; PIP₃, phosphatidylinositol 3,4,5-triphosphate; RA, rheumatoid arthritis; RLR, RIG-I-like receptor; RIG-I, retinoic acid-inducible gene I; SH3, Src homology 3; SHIP2, SH2 containing inositol polyphosphate 5-phosphatase-2; Tir, translocated intimin receptor; VASP, vasodilator-stimulated phosphoprotein; WASP, Wiskott-Aldrich syndrome protein; WAVE, WASP family verprolin-homologous protein; WH2, WASP-homology 2

Key words: IRTKS, I-BAR, cancer, membrane remodeling, insulin signaling

Subsequent research has shown that IRTKS functions as a scaffold protein with multiple domains, which results in diverse functions in a variety of cell activities. For example, IRTKS plays roles in regulating the formation of membrane protrusions; triggering pathogen-driven actin assembly; modulating insulin signaling, antiviral immunity and embryonic development; and promoting tumor occurrence and progression. It is also a candidate forensic biomarker of hypothermia. Nevertheless, a systematic summary of the biological functions of IRTKS and its underlying molecular mechanism is lacking. Therefore, the present review provides a comprehensive summary of the latest advancements in IRTKS research, thereby establishing a framework for understanding the contribution of IRTKS to diverse cell processes.

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1. Introduction

Insulin receptor (IR) tyrosine kinase substrate (IRTKS), also known as brain-specific angiogenesis inhibitor 1-associated protein 2 (BAIAP2)-like 1 (BAIAP2L1), was first identified as a phosphorylation substrate for the IR (1,2). The human IRTKS gene was initially cloned from endocrine organs (GenBank accession number, AF119666.2) and is located on chromosome 7 at q21.3-q22.1 (gene ID, 55971). The full-length human IRTKS is 109,441 bp (NC_000007.14) and includes 14 exons. Its coding region comprises 1,536 bp, which encodes a 511-amino acid protein (GenBank, AAF17223.2) with a molecular weight of ~57 kDa (1,3-5). Human IRTKS

is widely distributed in various tissues, with broad expression in the stomach, colon, duodenum, prostate, skin, thyroid and lung, and trace amounts in the spleen, ovary, brain and bone marrow (4).

2. I-BAR family

IRTKS is a member of the Bin-amphiphysin-Rvs (BAR) superfamily. The members of this family share the defining element of a BAR domain, which is a coiled structure that dimerizes into modules with membrane-binding and curvature-sensing abilities (6). The BAR domain protein was initially identified in the mammalian proteins Bin1 and amphiphysin, as well as the budding yeast Rvs167 protein (7). Based on crystal structures, the BAR domain superfamily includes several classes, including the classical BARs, the Fes/CIP4 homology-BARs (F-BARs) and the inverse-BARs (I-BARs) (8). These members differ in their effects on cell membrane remodeling (9). The classical BAR and F-BAR domains drive the formation of positive membrane curvature by forming banana-shaped α -helical dimers, which facilitate the formation of plasma membrane invaginations (8,10). However, the I-BAR domain forms a zeppelin-shaped structure that binds to phosphoinositide-rich membranes and generates negative membrane curvature in a phosphatidylinositol 4,5-bisphosphate-dependent manner, thereby inducing plasma membrane protrusions such as filopodia and lamellipodia (6,7).

There are five genes in the I-BAR family, namely IRSp53 (BAIAP2), missing-in-metastasis (MIM), IRTKS, FLJ22582 (also known as BAIAP2-like 2) and actin-binding protein ABBA, that encode homologous N-terminal sequences and were identified via human genome alignment (3). The N-terminal helical domains of the I-BAR proteins IRSp53 and MIM were the first shown to be evolutionarily conserved; hence, the I-BAR domain was originally named the IRSp53/MIM homology domain (IMD) (3,7).

In addition to the N-terminal IMD, the human I-BAR family proteins each possess a Wiskott-Aldrich syndrome protein (WASP)-homology 2 (WH2) domain in the C-terminus. The IRSp53, IRTKS and FLJ22582 proteins are distinctive in that they contain a canonical Src homology 3 (SH3) domain in the C-terminal half; therefore, these proteins are classified into the IRSp53 subfamily, primarily because IRSp53 was studied early and extensively. IRTKS and IRSp53 each have a WW domain-binding motif, which is in agreement with their close genetic relationship, with ~40.9% similarity (1,7) (Fig. 1). Therefore, the comparative study of these two proteins is a major topic in IRTKS research.

3. IRTKS and cellular morphology

A number of cellular processes, including cell migration, phagocytosis and axon pathfinding, are dependent on membrane deformation and cytoskeletal rearrangement, which are regulated by an array of signaling complexes that control actin assembly. As already noted, I-BAR domains, as sensors of membrane curvature, can induce membrane bending and anisotropic cytoskeletal rearrangement (1,7,10).

Cytoskeletal rearrangements involve multiple cell types and are regulated by various biological molecules.

Extracellular signals converge on small GTPases, which activate downstream effectors such as the WASP family verp-rolin-homologous protein (WAVE) complex, which stimulates the actin-related protein 2/3 (Arp2/3) complex. This complex nucleates new actin filaments, leading to the branching and crosslinking of preexisting filaments (11-13). Capping proteins bind to the barbed ends of filaments, which terminates filament growth and stabilizes the filament structure (14). By contrast, enabled (Ena)/vasodilator-stimulated phosphoprotein (VASP) proteins prevent filament capping, resulting in the elongation of actin filaments (15,16), and diaphanous-related formin 2 promotes fibrous actin (F-actin) elongation (17). In addition, numerous actin-bundling and crosslinking proteins are crucial for stabilizing actin filaments and for facilitating the extension of membrane projections at the cell periphery (18,19). IRSp53, which shares 40.9% sequence homology with IRTKS, interacts with Rho-GTPases Rac and cell division cycle 42 (Cdc42) (1). This interaction, in collaboration with various proteins, such as mammalian enabled, an Ena/VASP family member (20), the actin-bundling/capping protein known as epidermal growth factor receptor (EGFR) kinase substrate 8 (Eps8) (21), WAVE1/2 (22-24), mammalian diaphanous isoforms 1/2 (23,25) and dynamin 1 (26), promotes the formation of filopodia (1,4,27,28).

IRTKS-overexpressing HT1080 cells have been demonstrated to spread more than their parent cells, and to display F-actin clusters and the formation of lamellipodia, which demonstrates the role of IRTKS in cellular morphology (29). Studies have also shown that IRTKS plays a role in cytoskeletal rearrangement, membrane tubulation and the formation of protrusions, by I-BAR- and WH2 domain-dependent mechanisms (1,2,27,30).

Rho, Rac and Cdc42 are small GTPases of the Rho family, which play roles in the formation and rearrangement of actin stress fibers (31,32). Millard *et al* (1) revealed that IRTKS interacts with Rac, but not Cdc42, via its I-BAR domain, while its SH3 domain has an autoregulatory function in the control of I-BAR activity. Moreover, the ectopic expression of IRSp53 and IRTKS causes distinct changes in actin cytoskeletal organization. In COS7 cells, IRSp53 induces the formation of numerous long, wavy filopodia-like extensions, while low levels of IRTKS expression result in small actin microspikes at the cell periphery. By contrast, higher levels of IRTKS expression lead to the formation of clusters of short actin bundles around the cell periphery, rather than the development of filopodia (1,33).

Sudhaharan *et al* (27) established that the Rho in filopodia (Rif)-IRTKS-Eps8-WAVE2 pathway promotes the formation of dorsal filopodia and membrane ruffles via a coexpression system comprising IRTKS and its interacting partners in cancer cells. In this pathway, the Rho GTPase Rif interacts with IRTKS via its I-BAR domain in the GTP and GDP forms, and Eps8 and WAVE2 act as downstream modulators. Specifically, Eps8 reduces the size and increases the number of dorsal filopodia and membrane ruffles, while WAVE2 modulates the activity of dorsal membrane ruffling (Fig. 2) (27). In addition, the study reported that IRTKS did not interact with Cdc42, RhoA or Rac (27), which differs from the study by Millard *et al* (1), which reported that IRTKS binds with Rac.

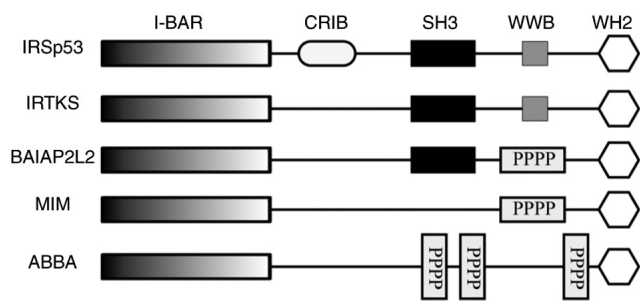


Figure 1. Structures of I-BAR family members. ABBA, actin-bundling protein with BAIAP2 homology; CRIB, Cdc42/Rac-interactive-binding domain; FLJ22582 (BAIAP2L2), brain-specific angiogenesis inhibitor 1-associated protein 2-like 2; I-BAR, inverse Bin-amphiphysin-Rvs domain; IRSp53, insulin receptor tyrosine kinase substrate p53; IRTKS, insulin receptor tyrosine kinase substrate; MIM, missing-in-metastasis; PPPP, proline-rich region; SH3, Src homology 3 domain; WH2, Wiskott-Aldrich syndrome protein homology 2 domain; WWB, WW binding domain.

Formins are multidomain proteins that act as actin polymerization factors. Formin-like 2 (FMNL2) is associated with filopodium formation in multiple cell types (34,35). Fox *et al* (30) recently identified IRTKS as a novel FMNL2-binding protein in melanoma cells, and proposed an updated hierarchical model based on the interdependence of IRTKS and FMNL2 in filopodia assembly. In this model, FMNL2 first docks to the plasma membrane via N-myristoylation and initiates membrane bending. IRTKS is then recruited to these membrane-bending sites and remains anchored to the membrane via its interaction with FMNL2. The two proteins mutually facilitate actin polymerization, leading to the initiation of filopodia assembly and growth (Fig. 2) (30).

Notably, the widely accepted notion that the outwardly curved I-BAR domain induces protrusions has also been challenged (36,37). Veltman *et al* (36) reported that in the amoeba *Dictyostelium*, the I-BARa protein, which contains a single I-BAR/SH3 domain similar to that of mammalian IRSp53 family proteins, is involved in clathrin-mediated endocytosis rather than the formation of actin-driven protrusions. This suggests that the physiological function of the I-BAR domain is not limited to protrusion formation, and further extends the functional areas of I-BAR domain proteins such as IRTKS.

4. IRTKS and pathogen-driven actin assembly

IRTKS and microvilli. Microvilli, also known as brush borders, are evolutionarily ancient cell surface protrusions that fulfill diverse functions in epithelial cells, enabling them to move and sense the surrounding environment. The growth, elongation, directional motility and collapse of microvilli are supported by actin bundles, with IRTKS and its binding partner Eps8 playing important roles in these processes (38–40). The two crucial proteins are located at the epithelial cell surface, where they mark future sites of microvillus growth (38). They together promote the elongation of microvilli (38) and help to maintain the directed motion of nascent microvilli during early differentiation (39); moreover, the sharp loss of Eps8 or IRTKS from the distal tip destabilizes nascent microvilli, leading to their collapse (40). Postema *et al* (38) suggested two

distinct mechanisms by which IRTKS promotes microvillar elongation: Eps8-dependent and Eps8-independent mechanisms. In the Eps8-dependent mechanism, IRTKS uses its SH3 domain to promote Eps8 enrichment at the microvillar tips to drive microvillar elongation, while in the Eps8-independent mechanism, IRTKS promotes elongation via a direct mechanism involving its actin-binding WH2 domain.

While a temporal molecular framework for understanding the assembly and dynamics of new microvilli is currently available, the mechanisms regulating the number of actin filaments per core bundle and the initial recruitment of distal tip-enriched factors to the plasma membrane remain unclear (40).

IRTKS and intestinal infections. Intestinal microvilli are tiny, finger-like projections on the apical end of enterocytes, that facilitate the digestion and absorption of nutrients, while also providing a barrier against luminal pathogens and toxins (38,41). The destruction of microvilli by pathogenic microbes can lead to nutrient malabsorption and osmotic imbalances and may even be life-threatening (42). Enterohemorrhagic *E. coli* (EHEC), particularly the O157:H7 serotype, is an important human pathogen causing diarrheal and systemic diseases. A hallmark of EHEC infections is epithelial attaching and effacing (A/E) lesions, which are characterized by microvilli effacement, actin assembly and the formation of ‘pedestal’ structures beneath the host cell membrane at sites of bacterial attachment (43).

A number of studies have shown that, by targeting the A/E lesion machinery, the EHEC serotype O157:H7 triggers intestinal colonization via two essential effector proteins, namely translocated intimin receptor (Tir) and *E. coli*-secreted protein F-like protein encoded on prophage U (EspFU) (33,44–46). ‘Type III’ secretion systems are complex bacterial structures that provide pathogenic bacteria with a unique virulence mechanism enabling them to inject bacterial effector proteins directly into host cells. These effector molecules manipulate host cells and contribute to a number of different infectious diseases (47,48). Through the ‘type III’ secretion system, Tir is translocated into host cells, localizes at sites of bacterial attachment, and initiates a signaling cascade upon binding to intimin, which leads to actin assembly and ‘pedestal’ formation.

IRSp53 and IRTKS play important roles in the pathogenesis of EHEC O157:H7 (49). The N-terminal I-BAR of IRTKS binds to Tir in a manner dependent on the Asn-Pro-Tyr 458 (NPY458) sequence of the latter; in addition, the C-terminal SH3 domain of IRTKS interacts with the C-terminal tandem PxxP motifs of EspFU, which results in EspFU recruitment (50,51). Therefore, IRTKS physically links the two EHEC effectors, Tir and EspFU (49–51). In host cells, the N-terminal repeat sequence of EspFU binds to the GTPase binding domain (GBD) of neuronal WASP (N-WASP). This activates N-WASP by destabilizing its interaction with an inhibitory protein. Subsequently, activated N-WASP stimulates the Arp2/3 actin nucleator complex, which promotes actin polymerization (Fig. 3).

In summary, IRTKS links Tir and the EspFU:GBD_{WASP}/N-WASP:Arp2/3 complex, which results in recruitment of the ternary complex and thus actin polymerization, subsequently generating ‘pedestals’ beneath bound bacteria (50–52).

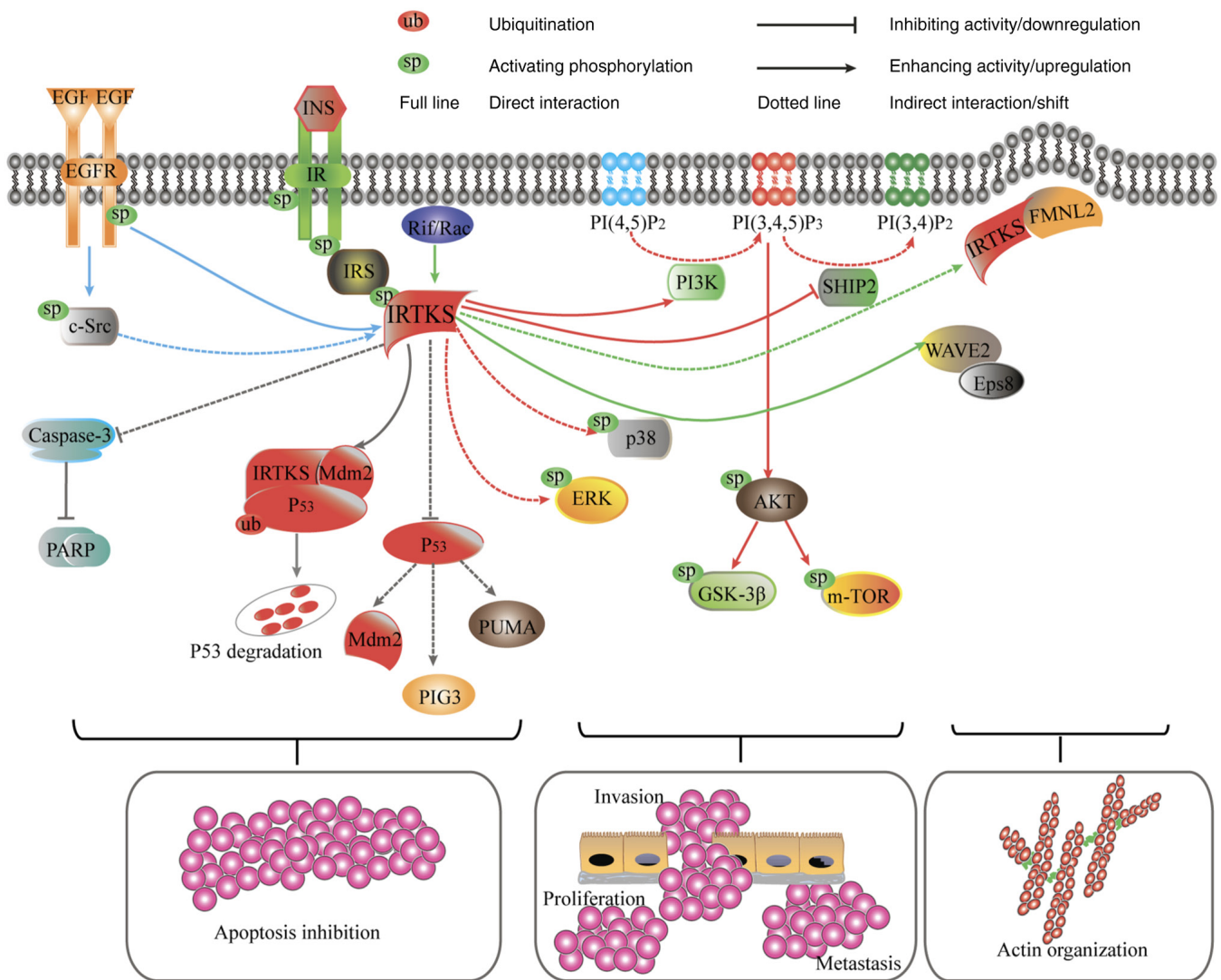


Figure 2. IRTKS-associated signaling pathways. IRTKS positively regulates IR-IRS-PI3K-AKT signaling. Overexpression of IRTKS increases the phosphorylation levels of ERK, p38, AKT, mTOR and GSK-3 β but attenuates SHIP2 activity (red lines). IRTKS inhibits the caspase-3-dependent apoptosis pathway and restricts the activity of p53, promotes IRTKS-Mdm2-p53 complex formation and thereby increases Mdm2-mediated p53 ubiquitination and degradation in unstressed cells (gray lines). IRTKS also initiates and maintains actin organization by interacting with Rif, Eps8, WAVE2 and FMNL2 (green lines). IRTKS activity is modulated by Src/EGFR (blue lines). EGF, epidermal growth factor; EGFR, EGF receptor; Eps8, EGFR kinase substrate 8; FMNL2, formin-like 2; GSK-3 β , glycogen synthase kinase-3 β ; INS, insulin; IR, insulin receptor; IRTKS, IR tyrosine kinase substrate; IRS, IR tyrosine kinase substrate; Mdm2, mouse double minute 2 homolog; mTOR, mammalian target of rapamycin; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; PI(3,4)/(4,5)P2, phosphatidylinositol 3,4,4,5-diphosphate; PI(3,4,5)P3, phosphatidylinositol triphosphate; PIG3, p53-inducible gene 3; PUMA, p53 upregulated modulator of apoptosis; Rif, Rho in filopodia; SHIP2, SH2 containing inositol polyphosphate 5-phosphatase-2; WAVE2, Wiskott-Aldrich syndrome protein family verprolin-homologous protein 2.

5. IRTKS and insulin signaling

Tyrosine phosphorylation of IRTKS in response to insulin stimulation was observed in COS-7 cells cotransfected with IRTKS and the IR β -subunit, which led to the initial identification of IRTKS as a substrate for the IR by Millard *et al* (1). These results suggest that IRTKS is associated with insulin signaling. However, following the identification of IRTKS in mammals, its role in regulating the formation of membrane protrusions and triggering pathogen-driven actin assembly attracted considerable attention, and less attention was given to its role in insulin signaling.

A study by Huang *et al* (53) found that IRTKS-knockout mice display characteristics of insulin resistance, including hyperglycemia, hyperinsulinemia and glucose intolerance, which can be

reversed by ectopic IRTKS expression. Furthermore, the study demonstrated that the phosphorylation of key molecules in the insulin signaling pathway, including IR, AKT and glycogen synthase kinase-3 β (GSK-3 β), is attenuated in response to IRTKS knockout or knockdown *in vivo* and *in vitro*, and this defective insulin signaling can be reversed by ectopic IRTKS. These findings indicate that IRTKS, as an adaptor of the IR, positively regulates insulin signaling (53). In addition, the study revealed that the expression of IRTKS in the livers of diabetic patients and mice is downregulated. Moreover, DNA hypermethylation of the human IRTKS promoter, which is associated with the downregulation of IRTKS expression in diabetes, was detected via bisulfite-treatment DNA sequencing (53). An increase in the phosphorylation level of extracellular regulated protein kinase (ERK) was also reported to occur following IRTKS overexpression (53).

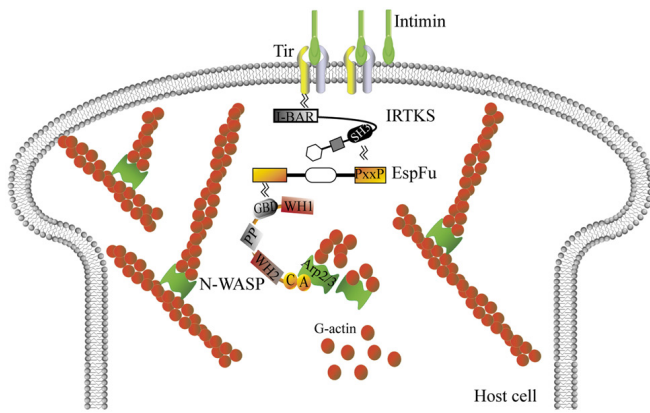


Figure 3. IRTKS initiates and maintains actin pedestal assembly in *E. coli* O157:H7. IRTKS links with the actin assembly effectors of *E. coli* O157:H7, including Tir, EspFU, WASP and Arp2/3. This leads to actin polymerization and subsequently generates 'pedestals' beneath bound bacteria. A, acidic peptide; ARP2/3, actin-related protein 2/3; C, connector region; EspFU, *E. coli*-secreted protein F-like protein encoded on prophage U; G-actin, globular actin; GBD, GTPase binding domain; I-BAR, inverse Bin-amphiphysin-Rvs; IRTKS, insulin receptor tyrosine kinase substrate; N-WASP, neuronal WASP; PP, proline-rich domain; SH3, Src homology 3 domain; Tir, translocated intimin receptor; WASP, Wiskott-Aldrich syndrome protein; WH1/2, WASP-homology 1/2 domain; WH2CA, WH2 connector acidic region.

SH2 containing inositol polyphosphate 5-phosphatase-2 (SHIP2) is a potent negative regulator of phosphatidylinositol 3-kinase (PI3K)-AKT signaling. In 2019, Wu *et al* (54) revealed that IRTKS interacts with SHIP2 in human liver cancer cell lines via the SH3 domain of IRTKS and the inositol polyphosphate-5-phosphatase catalytic domain of SHIP2. In addition, they demonstrated that phosphorylated active IRTKS attenuates the activity of SHIP2, and thereby suppresses the conversion of phosphatidylinositol 3,4,5-triphosphate (PIP₃) to phosphatidylinositol 3,4-bisphosphate, resulting in PIP₃ accumulation (54). The study also found that in addition to directly inhibiting SHIP2 activity, IRTKS overexpression also evokes the phosphorylation of AKT, mammalian target of rapamycin (mTOR) and GSK-3 β , which partially relieves the inhibitory effect of SHIP2 on insulin downstream molecules and promotes cancer cell proliferation (54) (Fig. 2).

These findings indicate that IRTKS, by promoting IR phosphorylation and inhibiting SHIP2 activity, modulates the IR-IRS-PI3K-AKT signaling pathway, which may affect glucose and lipid metabolism, and even cell growth and proliferation. Consequently, the expression and phosphorylation of IRTKS, the methylation status of the IRTKS gene, and IRTKS-interacting proteins, may have therapeutic potential against diabetes, cancer and related conditions.

6. IRTKS and tumors

IRTKS promotes the proliferation of tumor cells. There is a considerable quantity of literature reporting that IRTKS, as a scaffold protein, interacts with a variety of cancer-related gene products, thereby controlling cancer cell growth, differentiation, apoptosis and motility. The expression of IRTKS is upregulated in numerous types of human cancer tissues compared with that in adjacent normal tissues, including hepatocellular carcinoma (HCC), ovarian cancer (OC),

colorectal cancer (CRC), gastric cancer (GC) and pancreatic cancer (55-59). It has been shown that the knockdown of endogenous IRTKS inhibits the proliferation of these cancer cells, whereas the ectopic expression of IRTKS promotes their proliferation (54-59).

An integrative database analysis of IRTKS revealed that in OC tissues and cell lines, the level of IRTKS upregulation does not differ among various cell types, stages or grades of each histologic subtype (55). However, in HCC the upregulation of IRTKS expression is significantly associated with tumor volume and patient age, although not with sex, hepatitis B surface antigen status, differentiation or tumor-node-metastasis stage (58). In addition, IRTKS can interact with EGFR and positively regulate the EGFR-ERK signaling pathway in HCC cells, resulting in increased proliferation, which is also associated with an increase in the G1-to-S transition of the cell cycle (58). Another regulatory mechanism of IRTKS in HCC cells involves its interaction with SHIP2, which attenuates SHIP2 activity and leads to the accumulation of PIP₃, a substrate of SHIP2 (54). This process activates the phosphorylation of downstream insulin signaling molecules, including AKT, mTOR and GSK-3 β (Fig. 2). Consequently, this process also promotes cell proliferation (54).

In CRC cells, the overexpression of IRTKS promotes basic fibroblast growth factor-induced cell proliferation via the phosphorylation of AKT; however, no significant correlation was detected between IRTKS and SHIP2 via database analysis (55). However, other research has shown that IRTKS-overexpressing HCC cells do not exhibit changes in the phosphorylated AKT/AKT ratio compared with that in control HCC cells (58). Therefore, the mechanism by which IRTKS triggers cell proliferation remains unclear, and more studies with different cell types, stress factors or stress durations are required to elucidate the dynamic regulation of IRTKS-related signaling pathways.

IRTKS promotes tumor invasion and metastasis. Membrane fusion between tumor cells and neighboring tissues is a fundamental biological process of tumor cell invasion (60). A study revealed that in RAW264.7 macrophages, IRTKS is induced in response to stimulation with receptor activator of NF- κ B ligand, which contributes to osteoclastogenesis. In addition, the study indicated that IRTKS interacts with Talin domain-containing protein kinase substrate 5 (Tks5), a known regulator of invadopodia in cancer cells, via different SH3 sites on IRTKS (61). Therefore, IRTKS may drive osteoclast-osteoclast fusion as well as osteoclast-cancer cell fusion in concert with Tks5 (61).

Actin polymerization, cytoskeletal remodeling and membrane deformation are important for cell mobility (62). IRTKS is involved in actin dynamics and the development of membrane protrusions, which implies that IRTKS contributes to tumor invasion and metastasis. Notably, it has been observed that in cases of OC, the level of IRTKS expression in metastatic sites is higher than that in primary cancers (56). In HeLa cells, in addition to increasing the phosphorylation of ERK1/2 and p38 and activation of the small GTPases Rac1 and Cdc42, IRTKS also promotes the chemotactic response to serum and increases cellular polarity, as observed by an elongated cytoplasm and increased numbers of lamellipodia at the leading edges of the cells (63). Importantly, the SH3 domain of

IRTKS is key to the process of IRTKS-mediated cell migration associated with p38 phosphorylation and cytomembrane polarity (63).

Src functions as both a substrate and an upstream activator of receptor tyrosine kinases, which regulate various cellular functions, including adhesion, migration and invasion (64,65). Notably, it has been shown that IRTKS promotes the motility of HT1080 fibrosarcoma cells in a Src-dependent manner, with the tyrosine phosphorylation of IRTKS by c-Src or EGFR contributing to this process (Fig. 2) (65).

IRTKS suppresses the apoptosis of tumor cells. IRTKS is considered to act as an inhibitor of apoptosis during cancer progression. It prevents cell apoptosis through two key mechanisms: Inhibition of the caspase-3-dependent apoptosis pathway and suppression of p53 activity (56,57,66,67). The knockdown of IRTKS in OC cells treated with ultraviolet irradiation or cisplatin increases the protein levels of cleaved caspase-3 and poly (ADP-ribose) polymerase (56), which are key indicators of the execution phase of apoptosis (68). This suggests that IRTKS may protect cells from apoptosis by blocking the caspase-3-dependent apoptosis pathway (Fig. 2) (56).

p53 protects against genomic instability and oncogene expression via the induction of cell cycle arrest and apoptosis (69). Mouse double minute 2 homolog (Mdm2) regulates p53 levels and nuclear export in a concentration-dependent manner: High levels of Mdm2 promote p53 polyubiquitination and proteasomal degradation, whereas low levels of Mdm2 mediate the monoubiquitination and cytoplasmic localization of p53 (62). In unstressed cells with low levels of Mdm2, ectopic IRTKS inhibits the transactivating effect of p53 on its downstream genes, such as p53-inducible gene 3, p53 upregulated modulator of apoptosis and Mdm2. Simultaneously, IRTKS promotes the interaction between p53 and Mdm2, leading to the induction of p53 ubiquitination and cytoplasmic localization. Ultimately, IRTKS mediates apoptosis resistance under low-Mdm2 conditions (Fig. 2) (67). Furthermore, studies have suggested the existence of an IRTKS-Mdm2-p53 tertiary complex, and demonstrated that IRTKS overexpression promotes Mdm2-mediated p53 ubiquitination and degradation, suggesting a new cancer-promoting mechanism of IRTKS (57,67). However, when DNA damage occurs, IRTKS undergoes serine/threonine phosphorylation by checkpoint kinase 2 (57). This induces the dissociation of IRTKS from p53, and promotes the IRTKS-Mdm2 interaction (67). Subsequently, the binding of p53 to Mdm2 is blocked, which induces IRTKS ubiquitination and degradation but attenuates p53 ubiquitination and degradation, ultimately increasing p53-initiated DNA repair or apoptosis (57).

Notably, IRTKS overexpression has been shown to have no effect on the expression of p53, the phosphorylation and acetylation of certain amino acid residues of p53, and Mdm2-mediated p53 neddylation (67).

IRTKS fusion genes and tumors. Chromosomal translocations/rearrangements leading to kinase activation are important contributors to tumorigenesis, and IRTKS has been reported to undergo fusion to certain oncogenes.

Fibroblast growth factor receptor 3 (FGFR3) activation by mutation or overexpression is frequently detected in cases of

bladder cancer, and another mechanism of activation involves chromosomal rearrangements that generate constitutively activated fusion genes, such as the FGFR3-IRTKS fusion gene, which has been identified by several research groups (70-72). In 293T cells, the overexpression of IRTKS, as a fusion partner in FGFR3-IRTKS fusions, introduces dimerization motifs that activate FGFR fusion kinases (72). In addition, the stable expression of FGFR3-IRTKS in telomerase reverse transcriptase-expressing human mammary epithelial cell lines increases downstream ERK1/2 and STAT1 phosphorylation and promotes proliferation (72). Another study identified the FGFR3-IRTKS fusion gene in patients with bladder cancer and lung cancer, and demonstrated that FGFR3-IRTKS has potent tumorigenic activity. Specifically, it revealed that FGFR3-IRTKS not only activates growth signals, such as those generated by the mitogen-activated protein kinase pathway, but also inhibits tumor-suppressive signals, including those generated by the p53, retinoblastoma 1 and cyclin dependent kinase inhibitor 2A pathways (70). Williams *et al* (71) demonstrated a chromosomal translocation t(4;7) with breakpoints at FGFR3 on chromosome 4 and IRTKS on chromosome 7 in SW780 bladder cancer cells. All the aforementioned studies indicate that FGFR3-IRTKS-positive cells are sensitive to selective FGFR inhibitors (70-72). Therefore, the presence of a fusion gene may aid in the selection of patients for FGFR-targeted therapy.

Several other types of IRTKS gene fusion have been identified; for example, BRAF and IRTKS fusion has been detected by RNA sequencing in spitzoid melanoma (73), and an oncogenic mesenchymal MET and IRTKS fusion has been identified in papillary renal carcinoma, which results in constitutive activation of the MET kinase (74,75). Although these IRTKS-containing fusion genes have been shown to be oncogenic, the biology of gene fusion has not been characterized extensively, and responses to single-agent or combination IRTKS-directed targeted therapy are underexplored.

IRTKS and epigenetic reprogramming. In 2023, Cui *et al* (76) revealed for the first time that IRTKS can induce the deubiquitination of the histone methyltransferase SET domain bifurcated histone lysine methyltransferase 1 (SETDB1), thereby blocking proteasome-mediated SETDB1 degradation and allowing SETDB1 to accumulate through the recruitment of OUT deubiquitinase 4 (OTUD4). The upregulation of SETDB1 subsequently increases histone H3 lysine 9 trimethylation (H3K9me3), which leads to a reduction in chromatin accessibility. Accordingly, chromatin inaccessibility represses the transcription of numerous genes, including that encoding E-cadherin, which results in epithelial-mesenchymal transition and the metastasis of malignant cells (76). In addition, elevated IRTKS/SETDB1 levels in clinical tumor specimens have been found to be negatively associated with survival time (76). These results advance our understanding of the epigenetic reprogramming caused by IRTKS, and suggest that the IRTKS-OTUD4-SETDB1-H3K9me3 axis may constitute a new tumor therapeutic focus in the future.

Further findings from the same research team revealed that IRTKS localizes not only to the cell membrane and cytoplasm but also the nucleus under certain conditions. In addition, a noncanonical role of IRTKS as a nuclear protein was

identified, in which it regulates heterochromatin formation via liquid-liquid phase separation (77). The research also showed that IRTKS recruits ubiquitin-conjugating enzyme E2, small ubiquitin modifier 9 (Ubc9) to sumoylate heterochromatin protein 1- α (HP1 α), a critical factor for heterochromatin formation and maintenance, thereby stabilizing HP1 α . Also, IRTKS can integrate into and promote the phase separation of HP1 α , facilitating heterochromatin formation (77). By contrast, the absence of IRTKS leads to heterochromatin loss, increased global chromatin accessibility and the reactivation of repetitive DNA elements, which contributes to the enrichment of gene sets associated with cellular senescence and aging. The aforementioned molecular events accelerate cellular senescence and activate the cyclic GMP-AMP synthase-stimulator of interferon (IFN) genes pathway to trigger the senescence-associated secretory phenotype response (77). These discoveries indicate that IRTKS functions as an epigenetic regulator to stabilize heterochromatin architecture, which emphasizes the link between heterochromatin loss and cellular senescence. However, the underlying mechanism and whether IRTKS alleviates aging require further investigation.

Based on the findings of the present review, IRTKS amplifications and gene fusions have been recognized as drivers of oncogenesis. This knowledge promotes our understanding of carcinogenic mechanisms and may aid the identification of novel treatment targets associated with IRTKS.

The tumorigenic role of upregulated circRNA_102231, a noncoding RNA with a covalently closed ring structure, has been revealed in GC tissue (78). In addition, mechanistic analyses revealed that circRNA_102231 binds to IRTKS, thereby increasing IRTKS protein stability and promoting GC progression (78).

Stem cell therapy is currently being evaluated as a promising approach for oncotherapy. A study in which human amniotic mesenchymal stromal cells (hAMSCs) were cocultured with HT-29 colon cancer cells revealed that the hAMSC secretome restrains the growth and invasion of the HT-29 cells. This effect is mediated via the downregulation of EGFR/c-Src/IRTKS expression, as well as the reduced phosphorylation of p38/ERK1/2. Also, the secretome induces the apoptosis of HT-29 cells, as evidenced by the upregulation of Bax and downregulation of Bcl2 (66).

7. IRTKS and embryogenesis

Most studies on IRTKS function have focused on its role in adult tissues and cells, and investigation into its involvement in embryogenesis is limited. The embryos of IRSp53 knockout mice display pleiotropic phenotypes, including developmental delay and oligodactyly, and do not survive due to severely impaired cardiac and placental development (79). Although mice with IRTKS knockout alone do not exhibit developmental and phenotypical abnormalities, the concurrent deletion of IRSp53 and IRTKS results in exacerbated placental abnormalities, particularly affecting spongiotrophoblast differentiation and development, which results in an increased embryonic lethality ratio (53,79). Therefore, IRSp53 and IRTKS appear to have redundant genetic interactions in placental formation, with IRTKS being essential for proper placental development during embryogenesis (53,79).

8. IRTKS and antiviral immunity

IRTKS deficiency contributes to insulin resistance, which may play a role in clinical infections and immune regulations (77). In addition, an association between IRTKS and inflammation has been implicated in the disease progression of rheumatoid arthritis (RA), as evidenced by correlations between IRTKS and C-reactive protein, a routinely assessed marker of RA activity, in fibroblast-like synovial cells obtained from patients with RA (80).

In 2015, Xia *et al* (81) identified IRTKS as an inhibitory modulator of innate immune responses against RNA viruses. During RNA virus invasion, viral RNA is recognized primarily by RIG-I-like receptors (RLRs), a family of nucleic acid sensors that includes retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5) and laboratory of genetics and physiology 2 (82). RIG-I and MDA5 subsequently activate the mitochondrial adaptor protein mitochondrial antiviral signaling protein (MAVS). These events activate IFN regulatory factors and NF- κ B, which in turn stimulate the transcriptional induction of IFN-I/-III and other proinflammatory cytokines, thereby facilitating the eradication of RNA viruses (83).

Extracellular viral RNA triggers RLR activation, whereas a number of regulatory mechanisms are in place to prevent 'self-reactivity' and maintain the immune homeostasis of host RNA. The host RNA-binding protein poly(rC) binding protein 2 (PCBP2), a negative regulator of MAVS, has been shown to be involved in host cell mRNA stability (84). Xia *et al* (81) confirmed that IRTKS recruits Ubc9, an E2 ligase, to sumoylate PCBP2 at Lys37 in the nucleus, which causes PCBP2 to translocate to the cytoplasm. The cytoplasmic sumoylated PCBP2 initiates MAVS degradation, leading to the downregulation of host immune responses. The study demonstrated that IRTKS acts as an inhibitory regulator of the RIG-I-MAVS signaling pathway, and its deficiency augments the innate immune response of mice against RNA viruses, but not against DNA viruses or bacteria (81).

Therefore, IRTKS appears to be a negative modulator of antiviral immunity, which is crucial for balancing the inflammation induced by viral infection and preventing cell damage due to excessive inflammation.

9. IRTKS and hypothermia

Hypothermia, characterized by a core body temperature $<35^{\circ}\text{C}$, can be caused by excessive exposure to cold, drug poisoning, and metabolic or nervous system dysfunction (85). Although postmortem biochemical explorations have revealed several biomarkers of hypothermia, including catecholamines, cortisol, ketone bodies and free fatty acids, death by hypothermia remains a diagnosis by exclusion, as definitive and specific biomarkers are lacking (85,86).

Exposure to cold triggers a series of stress reactions in the body, including increased cortisol production and lipolysis, which specifically promotes ketogenesis to maintain the core temperature (85,87). Postmortem metabolomics has shown potential in providing valuable insights and improving diagnostic accuracy in cases of hypothermia. IRTKS-mediated insulin signaling strongly affects glucose and lipid metabolism,

suggesting that IRTKS may be a good candidate as a forensic biomarker of hypothermia. In a study of hypothermic mice continuously exposed to cold water compared with mice maintained under standard conditions, total of 4,094 differentially expressed genes were identified. Among them, IRTKS was the most downregulated, suggesting that it is a promising candidate biomarker of hypothermia (88).

As aforementioned, IRTKS deficiency contributes to insulin resistance, which involves various metabolic reactions in the body, including impaired glycolysis, increased lipolysis and consequently ketogenesis, which are similar to those associated with hypothermia induction and implies the relevance of IRTKS to hypothermia. However, further studies are required to establish the dependency relationships between IRTKS and hypothermia. In addition, issues associated with postmortem stability, the use of biological fluids, and the specificity and limitations of IRKS as a novel forensic biomarker of hypothermia require further investigation.

10. Conclusions and future prospects

IRTKS was initially identified as a substrate for the IR. While studies have shown that IRTKS positively regulates IR-IRS-PI3K-AKT signaling, little is known about the role of IRTKS in downstream pathways, molecular events and clinical disease manifestations.

Under physiological conditions, the IR-IRS-PI3K-AKT signaling pathway is triggered by various factors, including growth factors, cytokines and hormones, and mediates a number of cellular processes, such as cell proliferation, cell cycle regulation and metabolic homeostasis. Aberrant activation of the IR-IRS-PI3K-AKT pathway is commonly associated with the occurrence of cancers, metabolic disorders and autoimmune and inflammatory diseases, and the progression of multiple malignancies (89,90). Therapies that target specific cellular mechanisms, including tyrosine kinase inhibitors (TKIs), have shown great potential in the treatment of key human diseases such as hematological malignancies, solid tumors and autoimmune disorders (91,92). The tyrosine kinase family has emerged as one of the most important drug targets in the 21st century, with >60 small-molecule therapeutic drugs that target protein-tyrosine kinases being approved by the US Food and Drug Administration in 2024, and TKIs gaining prominence as effective pathway-directed agents (93).

Most small-molecule TKIs exert their effects by targeting and binding to the enzymatic domain and competitively blocking the ATP-binding pocket or inhibiting relevant signaling cascades. However, due to the complex cross-connections and compensatory mechanisms within signal transduction pathways, as well as the dynamic evolution of genetic profiles, single-target TKIs may not be clinically effective, and resistance to TKIs is emerging (94,95). Although treatments with TKIs have been demonstrated to prolong the overall survival of patients, unsatisfactory treatment efficiency, drug resistance and side effects limit their clinical application. Multikinase inhibitors that target several protein-tyrosine kinases simultaneously, or the combination of TKIs with other therapeutic approaches, such as radiation and chemotherapy, show improved outcomes in disease treatment (91). However,

multikinase inhibitors have both advantages and disadvantages. For example, sunitinib and cabozantinib have potent off-target effects on the Axl receptor protein tyrosine kinase, which can increase their clinical efficacy (93,96). However, the targeting of off-target kinases may also elicit unwanted adverse effects, such as hypertension, heart failure, and gastrointestinal and skin reactions (97). Accordingly, multikinase inhibition appears to be 'a double-edged sword', which makes the choice of the optimal treatment challenging (93).

Advancements in the understanding and monitoring of protein-tyrosine kinases and their associated signaling cascades could facilitate the development of new targeted therapies and improved clinical outcomes. Gaining insights into the biological and pathological functions of IRTKS may lead to the development of new approaches for the treatment or prevention of cancers and other diseases. Metabolic reprogramming is currently attracting considerable attention in the study of tumor pathogenesis and therapeutic development. Controlled by IRTKS, the conversion of metabolic efficiency, its direction, and the expression and activity of downstream effectors of IR-IRS-PI3K-AKT signaling, particularly key enzymes and transporters involved in metabolism, have emerged as promising areas for tumor research.

The role of IRTKS in various cell processes is of increased interest to researchers. Evidence suggests that IRTKS initiates and maintains membrane remodeling, induces the formation of protrusions, triggers pathogen-driven actin assembly, positively regulates insulin signaling, drives oncogenesis, participates in embryonic development, and negatively modulates antiviral immunity. The present review systematically summarizes advances in IRTKS research, providing insights into its role in disease pathogenesis and guiding the diagnosis and treatment of IRTKS-related diseases. These findings have both basic and clinical implications, expanding the range of therapeutic options. Understanding the regulatory mechanisms and signaling pathways associated with IRTKS is likely to remain an area of active research in the coming years.

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Authors' contributions

XZ contributed to acquisition, analysis, interpretation of the literature and drafted the manuscript. ZZ participated in revising the manuscript. Both authors read and approved the final version of the manuscript. Data authentication is not applicable.

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Not applicable.

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Competing interests

The authors declare that they have no competing interests.

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