Large and diverse numbers of human diseases with HIKE mutations

Francesca D. Ciccarelli, Angela Acciarito and Saverio Alberti+

Laboratory of Experimental Oncology, Department of Cell Biology and Oncology, Istituto di Ricerche Farmacologiche Mario Negri—Consortio Mario Negri Sud, 66030 Santa Maria Imbaro (Chieti), Italy

Received 2 February 2000; Accepted 7 February 2000

HIKE is a highly conserved sequence motif identified as a candidate pleckstrin-homology (PH) domain binding site in Gβ proteins, protein kinases, ankyrin and kinesin. HIKE motifs occur also in gelsolin, neurogranin, neuromodulin and in the PH domain of Bruton tyrosin kinase (BTK). Phosphatidylinositol-binding sequences more distantly related to HIKE are present in gelsolin, in the G protein-coupled receptor kinase 4 and in Trop-2. HIKE regions have been demonstrated to bind both proteins and lipids, and to regulate the interaction of Gβ, neuromodulin and the BTK PH domain with downstream effectors and the cell membrane. Remarkably, mutations of the HIKE regions are common in diverse human genetic diseases. Several HIKE mutations in protein kinases lead to constitutive activation and cellular transformation, e.g. in MEN-2B, acute myeloid and mast cell leukemias, hereditary papillary renal carcinomas and multiple myeloma. Kinase-inactivating HIKE mutations cause Hirschsprung’s disease, piebaldism, insulin resistance and developmental dysplasias. HIKE mutations in the PH domain of BTK lead to X-linked agammaglobulinemia, and different forms of amyloidosis are caused by mutations of HIKE-bearing molecules, for example gelsolin, Ret and Trop-2. Thus, quite diverse genetic diseases might share common molecular mechanisms. These include altered interactions of the mutated molecules with downstream effectors or the cell membrane, and defects in intracellular transport.

THE HIKE SEQUENCE MOTIF

HIKE is a highly conserved sequence motif that was originally identified as a candidate binding site for pleckstrin-homology (PH) domains in the β subunit of trimeric G proteins (Gβ), in the activation loop of kinases, in ankyrin and in kinesin (1,2). Comparison of the HIKE consensus with other known phosphatidylinositol (PI)-binding sites identifies HIKE-like PI-binding sequences in gelsolin, an actin filament severing and capping protein, in the related proteins villin and severin, in neurogranin and neuromodulin and in PLCγ1 (3–10) (Fig. 1A). A more divergent HIKE sequence is present in the PH domain of the Bruton tyrosin kinase (BTK) (1,2).

Specific charged residues and their spacing are characteristically conserved in the HIKE subset of PI-binding sequences (1–5,9). Other conserved characteristics are the overall abundance of bulky hydrophobic residues flanking the charged amino acids, and the good conservation of an H residue N-terminal to the most conserved K. HIKE sequences also share a common structure, i.e. a β strand–loop–β strand configuration (1,11) (Fig. 2). The two β strands are largely conserved in length, but can be orientated at largely different angles, i.e. from widely open to essentially parallel. HIKE-like sequences are commonly exposed to solvent and are located either in clefts of the molecule or on its surface. Interestingly, their accessibility to binding can be regulated by conformational changes of the bearing molecule (12). The β strand–loop–β strand configuration is conserved even in the more divergent members of the family, suggesting a critical selective pressure for function.

These characteristics have allowed the identification of more distantly related members of the HIKE family in Trop-2 (13,14), a calcium signal transducer overexpressed by tumor cells (15), in the G protein-coupled receptor kinase 4 (GRK4) and in gelsolin (13) (Figs 1–3).

In this review, these PI-binding sequences will be collectively referred to as HIKE regions.

FUNCTIONAL ROLE OF THE HIKE REGION AS AN INTERACTIVE BINDING SITE FOR PROTEINS AND LIPIDS

Binding of PIs to HIKE regions has been demonstrated for several proteins, for example gelsolin, villin, severin, neurogranin, neuromodulin, GRK4, PLCγ1 (3–10,16) and the PH domain of BTK (1,2,17). The HIKE region of Gβ is also likely to bind phospholipids (2). The N-terminal acyl chain of Gα and the C-terminal prenyl group of Gγ anchor the Gαβγ trimer to the cell membrane (18,19). These lipid-modified sites of Gα and Gγ are located in a common face of the heterotrimer, that constitutes the membrane-docking site of G proteins (19). Remarkably, the core of the HIKE region lies between the Gα and Gγ membrane anchors, it closely faces the cell membrane and probably directly binds to it. It would be interesting to determine whether the HIKE regions in the activation loop of

+To whom correspondence should be addressed. Tel: +39 0872 570 293; Fax: +39 0872 578 240; Email: alberti@cmns.mnegri.it
protein kinases (12, 20), or in the cytoplasmic region of Trop-2, also bind to lipids.

HIKE regions specifically bind to proteins. Indeed, mutagenesis of specific HIKE residues abolishes the binding of Gβ to Gα and Gγ, and to downstream effectors, for example the β-adrenergic receptor kinase, adenyl cyclase 2, phospholipase C β2, inward rectifier K channels, calcium channel α1B, calmodulin and phosducin (2). Mutagenesis of the HIKE region of neuromodulin abolishes the binding to calmodulin and PKC (7). The activation loop of protein kinases binds substrate peptides, and residues in the HIKE region, and near to it, are critical to this function (1, 11, 12, 21, 22). The HIKE region of Trop-2 binds PKC and is phosphorylated by it (23). In addition, the cytoplasmic tail of Ep-CAM/Trop-1 (24, 25), a structural and functional homologue of Trop-2 (13, 14), binds to α-actinin (26), and Trop-2 might also bind to it. Finally, PH domains have also been demonstrated to bind to proteins (1, 2, 27–29), although it is unclear whether the HIKE region is involved in this process.

Mutagenesis of specific HIKE residues affects the assembly of Gα with Gβγ, alters the GTP exchange rate of Gα (1, 2), and causes a paradoxical increase in the binding of β-ARK, PLC-β2 and CCo1B to the Gβ HIKE (30). These findings suggest that HIKE plays a regulatory role in protein–protein binding. The binding of calmodulin to the Gβ and the neuromodulin HIKE (7, 31, 32), and the phosphorylation of the neuromodulin and Trop-2 HIKE by PKC (7, 23) are consistent with a regulatory role of the HIKE region. Calmodulin is, indeed, a widespread regulator of inter-molecular interactions (33), and PKC regulates the binding of downstream effectors to target sequences on phosphorylation at S/T residues (12, 34).

**Figure 1.** Sequence of the HIKE core regions in different classes of molecule. (A) Canonical HIKE motifs, as in Gβ5, PKCδ and Akt, and HIKE-like PI-binding sequences, i.e. villin, severin, gelsolin, PLCγ1 and BTK. (B) PI-binding consensus sequences that display sequence and structure similarities with the HIKE motif. Red, positively charged residues; blue, negatively charged amino acids; green, bulky hydrophobic residues.

**Figure 2.** Three-dimensional structures of molecules that contain HIKE regions. (A) Insulin receptor kinase catalytic domain; (B) G protein β subunit; (C) PLCδ1 PH domain, as a model of the BTK PH domain; (D) gelsolin. Structures are displayed as ribbons using the RasMol program. HIKE regions are in blue. Both HIKE-like PI-binding regions of gelsolin are highlighted.
Interestingly, the binding of calmodulin and PI over the HIKE regions of neurogranin and neuromodulin is interactive, and is modulated by phosphorylation by PKC (7). Analogously, PH domains bind more efficiently to Gβ in the presence of PI (10). Thus, simultaneous binding of lipids and proteins to the HIKE region can occur, and modulates its overall affinity for the binding molecules.

In summary, HIKE appears to be a critical regulatory region for both protein–protein and protein–lipid interactions (2). It is tempting to speculate that the conserved features of the HIKE regions are determined by this common function, whereas its variant features, for example the angle between the two β strands, the relative order of specific charged versus hydrophobic residues and their spacing, may confer target specificity.

MUTATIONS OF THE HIKE REGION IN HUMAN GENETIC DISEASES

The findings summarized above demonstrate that HIKE regions are important regulatory sites along pathways of signal transduction or in cytoskeletal proteins. This has made HIKE sites attractive candidates for the occurrence of mutations that could cause human genetic diseases. The HIKE regions of different proteins were analysed for the presence of disease-causing mutations. Remarkably, these were found to be frequent and widespread (Table 1; OMIM databank at http://www.ncbi.nlm.nih.gov/htbin-post/Omim/).

HIKE MUTATIONS IN PROTEIN KINASES

The R897Q mutation in the HIKE region of Ret, a receptor tyrosine kinase expressed in neural crest-derived ganglion cells, causes Hirschsprung’s disease (35) or aganglionic megacolon, a congenital disorder characterized by the absence of enteric ganglia. On the other hand, the A883F and M918T mutations generate a dominant transforming gene, and cause multiple endocrine neoplasias (MEN) type 2B, sporadic medullary thyroid carcinomas (MTC) and intestinal ganglion-neuromatosis (36–39). The R791G and G812V mutations of Kit cause piebaldism, a pigmentation defect that is demonstrated by a white forelock and heterochromia iridies. Similarly to patients with Ret mutations, patients affected by piebaldism have functional gut abnormalities and megacolon, and mice with W mutations at the Kit locus also lack myenteric plexus cells. On the other hand, mutations of D816 and D820 activate the transforming ability of Kit and cause acute myeloid and mast cell leukemias (40,41). Remarkably, most of the Met mutations that cause hereditary papillary renal carcinomas (HPRC) occur in the HIKE region (42). Several mutations of the insulin receptor α chain (IRα) cause insulin resistance or frank diabetes mellitus and acanthosis nigricans (43), a skin disorder characterized by thickening and hyperpigmentation of the flexural areas. The mutation of the FGFR-3 K650 to E causes thanatophoric dwarfism, a common lethal skeletal dysplasia (44), whereas a mutation of the same residue to M induces oncogenic activation and causes multiple myeloma (45). The S227A mutation of Rsk-2 (S6-kinase) is associated with the Coffin–Lowry syndrome, an X-linked disorder characterized by mental retardation, faciodigital dysmorphism and skeletal deformation (46).

HIKE MUTATIONS IN THE PH DOMAIN OF BTK

The F25, R28 and T33 mutations in the HIKE region of the BTK PH domain cause X-linked agammaglobulinemia (XLA), a humoral immunodeficiency that is demonstrated by maturation defects of B lymphocytes (47). Analogously, the R28C and E41K mutations cause Xid in mice. Remarkably, E41K also causes the oncogenic activation of BTK and induces B lymphocyte hyperproliferation (48). XLA-causing mutations are spread over the entire length of the BTK sequence (47), and commonly inactivate the kinase. On the other hand, mutations in the HIKE region of BTK prevent the recruitment of the kinase to the cell membrane (49), without inactivating its catalytic activity. Moreover, at least one mutation in this region, i.e. E41K, hyperactivates the kinase and furnishes an oncogenic potential. Thus, mutations of the BTK HIKE appear to cause disease through an alteration of a regulatory function.

HIKE MUTATIONS IN AMYLOIDOTIC DISEASES

Gelsolin contains two regions that bind PI, one from residue 135 to 142 and the second one from residue 161 to 175 (6,11) (Figs 1–3). Both regions display a β strand–loop–β strand configuration, and are arranged side-by-side to form a single β sheet (Figs 2 and 3). The gelsolin D187 binds to the conserved K166 of HIKE (11). Mutations of D187 to N or Y disrupt this
interaction and result in an altered structure of this region, and in abnormal degradation of the mutated gelsolin.

Interestingly, mutations affecting the MIS1/TROP2 gene have been demonstrated recently to cause the gelatinous drop-like corneal dystrophy, that represents the type III familial amyloidosis (50). The mutations that have been identified in Japanese families affected by the disease are stop codons or large deletions, and cause the loss of the cytoplasmic PI-binding consensus region. Although it is unclear whether a secreted form of soluble Trop-2 is produced and plays a role in the disease, an accumulation of the mutant molecule in the Golgi apparatus because of defective transport has been demonstrated (50).

An altered binding of the X11 PTB domain to the β-amyloid precursor protein may play a role in Alzheimer’s amyloidosis (51). The MEN-2A syndrome is caused by mutations of the HIKE-bearing RET gene, and demonstrates pheochromocytomas, amyloid-rich MTC and cutaneous lichen amyloidosis (52). Moreover, Ret mutations in the HIKE region cause MEN-2B and sporadic MTC (36).

Taken together, these findings indicate that different forms of amyloidosis can be caused by mutations of molecules that bear HIKE regions, or other PTB/PH domain targeting sequences. These alterations are correlated with an abnormal intracellular transport and secretion of the affected protein, and this might be directly linked to the pathologic manifestations of the disease.

**FUNCTIONAL CONSEQUENCES OF HIKE MUTATIONS AT THE MOLECULAR LEVEL**

Mutations in the HIKE region cause either loss-of-function or constitutive activation of the bearing molecule, consistent with a regulatory role of this region (Table 1). The K650E mutation of FGFR-3 causes constitutive activation of the kinase in the absence of ligand and of tyrosine autophosphorylation (44,53). The M918T mutation of Ret causes an increased autophosphorylation of the kinase and alters its substrate specificity (39). The D816V mutation of Kit similarly causes kinase activation and increased tyrosine autophosphorylation (40,54).

Removal of Y1248 upregulates the kinase activity of Met (55), whereas it is the phosphorylation of the nearby Y1232 and Y1233 that activates the kinase and transforms cells (56). The three Y autophosphorylation sites of IRα (57), i.e. Y1185, Y1186 and Y1187, are phosphorylated in insulin resistance and acanthosis nigricans, and R1191Q is involved in non-insulin-dependent diabetes mellitus (NIDDM).

The K650E mutation of S6-kinase causes constitutive activation of the kinase and alters its substrate specificity (46). Similarly, the BTK V41K mutation activates the kinase and transforms cells (48).

**Table 1.** Germline and somatic mutations of the HIKE region in human genetic diseases

<table>
<thead>
<tr>
<th>Molecule (accession no.)</th>
<th>HIKE residues</th>
<th>Mutations</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ret (U05016) 857–911</td>
<td>A883F</td>
<td>MEN-2B and MTC (36,38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R897Q</td>
<td>Hirschsprung’s disease (35)</td>
<td></td>
</tr>
<tr>
<td>Kits (P10721) 775–829</td>
<td>R791G</td>
<td>Piebaldism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G812V</td>
<td>Piebaldism</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D816V</td>
<td>Mast cell leukemia (40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D816Y</td>
<td>Acute myeloid leukemia (41)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D820G</td>
<td>Aggressive mastocytosis (41)</td>
<td></td>
</tr>
<tr>
<td>Met (P08581) 1205–1261(^a)</td>
<td>V1206L</td>
<td>HPRC (42)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L1213V</td>
<td>HPRC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>V1238I</td>
<td>HPRC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D1246</td>
<td>HPRC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y1248</td>
<td>HPRC</td>
<td></td>
</tr>
<tr>
<td>IRet (P06213) 1142–1196</td>
<td>A1161T</td>
<td>Insulin resistance (57)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>A1162E</td>
<td>Insulin resistance and acanthosis nigricans</td>
<td></td>
</tr>
<tr>
<td></td>
<td>M1180I</td>
<td>Insulin resistance</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R1191Q</td>
<td>NIDDM</td>
<td></td>
</tr>
<tr>
<td>FGFR-3 (A38576) 600–654</td>
<td>K650E</td>
<td>Thanatophoric dysplasia (44)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>K650M</td>
<td>Multiple myeloma (45)</td>
<td></td>
</tr>
<tr>
<td>S6-kinase (P23443) 176–230</td>
<td>S227A</td>
<td>Coffin–Lowry syndrome (46)</td>
<td></td>
</tr>
<tr>
<td>BTK (Q06187) 25–45(^b)</td>
<td>F25</td>
<td>XLA (47)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>R28</td>
<td>XLA and Xid</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T33</td>
<td>XLA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E41K</td>
<td>Cell transformation and Xid (48)</td>
<td></td>
</tr>
<tr>
<td>Gelsolin (P06396) 155–175(^bc)</td>
<td>D187</td>
<td>Familial amyloidosis (Finnish type) (65)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)The numbering is as in ref. 42.

\(^b\)HIKE core region (1).

\(^c\)The numbering is as in ref. 11.
COMMON FEATURES OF THE SIGNAL TRANSDUCTION AND CYTOSKELETAL GENETIC DISEASES CAUSED BY HIKE MUTATIONS

Different diseases caused by mutations of HIKE regions in kinases, cytoskeletal components and PH domains may demonstrate the common theme of an altered regulation of the mutated molecule, as opposed to a gross functional inactivation. An example is that of XLA, an immunodeficiency caused by mutations of the BTK. XLA-causing mutations are found over the entire BTK gene (47), and commonly inactivate the enzymatic activity of the kinase. On the other hand, mutations of the BTK PH domain that induce loss of function are not deleterious to the kinase catalytic activity, but act by preventing the recruitment to the cell membrane (49). Mutations also exist, for example E41K, that cause XLA by inducing a constitutive hyperactivation of the BTK kinase (47).

Several other hyperactivating mutations of the HIKE regions are demonstrated and lead to cellular transformation, for example in MEN-2B, MTC, intestinal ganglioneuromatosis, acute myeloid and mast cell leukemias, hereditary papillary renal carcinomas and multiple myeloma (36,38,40–42,45). A striking feature of these mutations is that they lie quite close to, or even overlap with, mutations that inactivate the function of the bearing molecule. Examples are: the occurrence of ganglionic megacolon versus intestinal ganglioneuromatosis in patients bearing mutations of the Ret HIKE (35,37), or the occurrence of connective tissue dysplasia (44) versus multiple myeloma (45) on mutation of the same K650 residue of FGFR-3 to E or M, respectively (Table 1).

Diseases caused by HIKE mutations might also share an altered transport and degradation of the mutation-bearing molecule. An example is that of amyloidotic diseases (36,50–52,65), but this is not exclusive to the latter, since analogous mechanisms can be affected by Kit (54) or ZAP-70 mutations (60–62).

The selective consequences of HIKE mutations also are consistent with a model of an altered regulatory function. For example, several inactivating or oncogenic mutations of Ret, that cause Hirschsprung’s disease or MEN-2A, respectively, are spread over different regions of the molecule. On the other hand, MEN-2B and intestinal ganglioneuromatosis are only caused by mutations that occur near the HIKE region, for example A883F and M918T (36–39). Analogously, oncogenic activating mutations only occur in the HIKE region of BTK (47) (see above).

CONCLUSIONS

A structural and functional analysis of HIKE mutations in different signal transduction and cytoskeletal human diseases suggests that their pathogenesis might demonstrate common molecular mechanisms, for example a perturbed interaction with downstream effectors or with the cell membrane, and an altered intracellular transport. This might explain unexpected similarities in the clinical appearance of different diseases, for example the occurrence of aganglionic megacolon in patients with either Ret- or Kit-inactivating mutations (35). It may also bear relevance for the development of parallel therapeutic approaches for these diseases.

ACKNOWLEDGEMENTS

This work has been supported by the Italian Association for Cancer Research (AIRC) and the National Research Council (CNR—Convenzione Consorzio Mario Negri Sud).

REFERENCES


5. Barnes, I.A. and Gomes, A.V. (1995) PEST sequences in calmodulin-


