# Examples from BioCreative VI Precision Medicine Track Corpus – abstract excerpts identifying PPI affected by mutations

Paper: Overview of the BioCreative VI Precision Medicine Track: Mining protein interactions and mutations for precision medicine

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**Abstract**

This document lists 10 random abstracts from the BioCreative VI Precision Medicine Track corpus. For each document we list the PMID, the pair of interacting genes as Entrez Gene identifiers (as curated by curators) and number of teams that have extracted that relation correctly. We follow by the title and abstract of the PubMed document. The highlighted text was marked by curators as evidence for the relation. The relation is not always expressed in one sentence.

**PMID**:15700267 **RELATION**:1398\_1793 **FREQ**:13

**C-terminal SH3 domain of CrkII regulates the assembly and function of the DOCK180/ELMO Rac-GEF.**

**Abstract**

Genetic studies in Caenorhabditis elegans identified an evolutionarily conserved CED-2 (CrkII), CED-5 (DOCK180), CED-12 (ELMO), CED-10 (Rac1) module important for cell migration and phagocytosis of apoptotic cells. Previous studies have shown that DOCK180 and ELMO comprise an unconventional bipartite Dbl homology domain-independent Rac guanine nucleotide exchange factor (Rac-GEF); but it is still unclear how CrkII functions in Rac-GEF activity. In this study, we have characterized a unique function of CrkII in phagocytosis and Rac activation mediated by the C-terminal SH3 domain, a region of CrkII that has no clear cellular or biochemical function. We found that mutations that disrupt the C-terminal SH3 domain of CrkII (CrkII-SH3-C) abrogate engulfment of apoptotic cells and impair cell spreading on extracellular matrix. Surprisingly, despite the effects on engulfment, W276K CrkII strongly potentiated Rac-GTP loading when ectopically expressed in HEK 293T cells. Contrary to the effects of the true dominant negative SH2 domain mutants (R38K CrkII) and SH3-N domain mutants (W170K CrkII) that prevent macromolecular assembly of signaling proteins, W276K CrkII increases association between DOCK180 and CrkII as well as constitutive tethering of the Crk/DOCK180/ELMO protein complex that interacted with RhoG. Our results indicate that while N-terminal SH3 of CrkII promotes assembly between CrkII and DOCK180, the C-terminal SH3 of CrkII regulates the stability and turnover of the DOCK180/ELMO complex. Studies with W276K CrkII may offer a unique opportunity to study the structure and function of the DOCK180/ELMO Rac-GEF.

**PMID**:16969499 **RELATION**:672\_7157 **FREQ**:13

**Missense mutations of BRCA1 gene affect the binding with p53 both in vitro and in vivo.**

**Abstract**

Women with BRCA1 gene mutations have an increased risk for breast and ovarian cancer (BOC). Classification of missense variants as neutral or disease causing is still a challenge and has major implications for genetic counseling. BRCA1 is organized in an N-terminal ring-finger domain and two BRCT (breast cancer C-terminus) domains, involved in protein-protein interaction. The integrity of the C-terminal, BRCT repeat region is also critical for BRCA1 tumor suppressor function. Several molecular partners of BRCA1 have so far been identified; among them, the tumor suppressor protein p53 seems to play a major role. This study was aimed at evaluating the impact of two missense mutations, namely the W1837R and the S1841N, previously identified in BOC patients and located in the BRCT domain of the BRCA1 gene, on the binding capacity of this protein to p53. Co-immunoprecipitation assays of E. coli-expressed wild-type and mutated BRCTs challenged with a HeLa cell extract revealed, for the S1841N variant a significant reduction in the binding activity to p53, while the W1837R mutant showed an inverse effect. Furthermore, a clonogenic soft agar growth assay performed on HeLa cells stably transfected with either wild-type or mutant BRCA1 showed a marked decrease of the growth in wild-type BRCA1-overexpressing cells and in BRCA1S1841N-transfected cells, while no significant changes were detected in the BRCA1W1837R-transfected cells. These results demonstrate that: i) distinct single nucleotide changes in the BRCT domain of BRCA1 affect binding of this protein to the tumor suppressor p53, and ii) the two missense mutations here described are likely to play a role in breast tumorigenesis. We suggest that in vitro/in vivo experiments testing the effects of unclassified BRCA1 gene variants should therefore be taken in to consideration and that increased surveillance should be adopted in individuals bearing these two BRCA1 missense alterations.

**PMID**:11463845 **RELATION**:1026\_207 **FREQ**:5

**Akt-dependent phosphorylation of p21(Cip1) regulates PCNA binding and proliferation of endothelial cells.**

**Abstract**

The protein kinase Akt is activated by growth factors and promotes cell survival and cell cycle progression. Here, we demonstrate that Akt phosphorylates the cell cycle inhibitory protein p21(Cip1) at Thr 145 in vitro and in intact cells as shown by in vitro kinase assays, site-directed mutagenesis, and phospho-peptide analysis. Akt-dependent phosphorylation of p21(Cip1) at Thr 145 prevents the complex formation of p21(Cip1) with PCNA, which inhibits DNA replication. In addition, phosphorylation of p21(Cip1) at Thr 145 decreases the binding of the cyclin-dependent kinases Cdk2 and Cdk4 to p21(Cip1) and attenuates the Cdk2 inhibitory activity of p21(Cip1). Immunohistochemistry and biochemical fractionation reveal that the decrease of PCNA binding and regulation of Cdk activity by p21(Cip1) phosphorylation is not caused by altered intracellular localization of p21(Cip1). As a functional consequence, phospho-mimetic mutagenesis of Thr 145 reverses the cell cycle-inhibitory properties of p21(Cip1), whereas the nonphosphorylatable p21(Cip1) T145A construct arrests cells in G(0) phase. These data suggest that the modulation of p21(Cip1) cell cycle functions by Akt-mediated phosphorylation regulates endothelial cell proliferation in response to stimuli that activate Akt."

**PMID**:9234717 **RELATION**:12402\_18595 **FREQ**:4

**Phosphotyrosine binding domain-dependent upregulation of the platelet-derived growth factor receptor alpha signaling cascade by transforming mutants of Cbl: implications for Cbl's function and oncogenicity.**

**Abstract**

Recent studies have demonstrated that Cbl, the 120-kDa protein product of the c-cbl proto-oncogene, serves as a substrate of a number of receptor-coupled tyrosine kinases and forms complexes with SH3 and SH2 domain-containing proteins, pointing to its role in signal transduction. Based on genetic evidence that the Caenorhabditis elegans Cbl homolog, SLI-1, functions as a negative regulator of the LET-23 receptor tyrosine kinase and our demonstration that Cbl's evolutionarily conserved N-terminal transforming region (Cbl-N; residues 1 to 357) harbors a phosphotyrosine binding (PTB) domain that binds to activated ZAP-70 tyrosine kinase, we examined the possibility that oncogenic Cbl mutants may activate mitogenic signaling by deregulating cellular tyrosine kinase machinery. Here, we show that expression of Cbl-N and two other transforming Cbl mutants (CblY368 delta and Cbl366-382 delta or Cb170Z), but not wild-type Cbl, in NIH 3T3 fibroblasts leads to enhancement of endogenous tyrosine kinase signaling. We identified platelet-derived growth factor receptor alpha (PDGFR alpha) as one target of mutant Cbl-induced deregulation. In mutant Cbl transfectants, PDGFR alpha was hyperphosphorylated and constitutively complexed with a number of SH2 domain-containing proteins. PDGFR alpha hyperphosphorylation and enhanced proliferation of mutant Cbl-transfected NIH 3T3 cells were drastically reduced upon serum starvation, and PDGF-AA substituted for the maintenance of these traits. PDGF-AA stimulation of serum-starved Cbl transfectants induced the in vivo association of transfected Cbl proteins with PDGFR alpha. In vitro, Cbl-N directly bound to PDGFR alpha derived from PDGF-AA-stimulated cells but not to that from unstimulated cells, and this binding was abrogated by a point mutation (G306E) corresponding to a loss-of-function mutation in SLI-1. The Cbl-N/G306E mutant protein, which failed to induce enhanced growth and transformation of NIH 3T3 cells, also failed to induce hyperphosphorylation of PDGFR alpha. Altogether, these findings identify a novel mechanism of Cbl's physiological function and oncogenesis, involving its PTB domain-dependent direct interaction with cellular tyrosine kinases."

**PMID**:16144832 **RELATION**:300772\_60590 **FREQ**:0

**Pias1 interaction and sumoylation of metabotropic glutamate receptor 8.**

This relation (Pias1-mGluR8-C) has not been extracted from any of the teams when looking at the exact match. Several teams predicted relations containing the Pias1 gene (mapped to the mouse, human and one team successfully mapped it to the correct organism rat). Similarly for the mGluR8-C gene. However, when checking the predicted relations, the organisms were all mismatched.

After correcting with the Homologene IDs, we found one team had paired the rat gene with the human gene, one team had paired the mouse gene with the human gene, and one team had used both human gene identifiers.

We think the difficulty in identifying this relation could be due to two reasons: 1. Mapping genes to correct organism is a very difficult problem, and 2. The mutation described in the (highlighted) sentence explaining the interaction may not have been picked up by the teams.

**Abstract**

Group III presynaptic metabotropic glutamate receptors (mGluRs) play a central role in regulating presynaptic activity through G-protein effects on ion channels and signal transducing enzymes. Like all Class C G-protein-coupled receptors, mGluR8 has an extended intracellular C-terminal domain (CTD) presumed to allow for modulation of downstream signaling. In a yeast two-hybrid screen of an adult rat brain cDNA library with the CTDs of mGluR8a and 8b (mGluR8-C) as baits, we identified sumo1 and four different components of the sumoylation cascade (ube2a, Pias1, Piasgamma, Piasxbeta) as interacting proteins. Binding assays using recombinant GST fusion proteins confirmed that Pias1 interacts not only with mGluR8-C but also with all group III mGluR CTDs. Pias1 binding to mGluR8-C required a region N-terminal to a consensus sumoylation motif and was not affected by arginine substitution of the conserved lysine 882 within this motif. Co-transfection of fluorescently tagged mGluR8a-C, sumo1, and enzymes of the sumoylation cascade into HEK293 cells showed that mGluR8a-C can be sumoylated in vivo. Arginine substitution of lysine 882 within the consensus sumoylation motif, but not other conserved lysines within the CTD, abolished in vivo sumoylation. Our results are consistent with post-translational sumoylation providing a novel mechanism of group III mGluR regulation."

**PMID**:8623535 **RELATION**:1489075\_1489080 **FREQ**:0

**Domains of the E1 protein of human papillomavirus type 33 involved in binding to the E2 protein.**

We think the difficulty in identifying this relation could be because the interaction and the effect of the mutation are in two different sentences.

**Abstract**

Papillomavirus E1 and E2 proteins are essential for the initiation of viral DNA replication. We have now analyzed the interaction of E1 and E2 of human papillomavirus type 33, which is associated with cervical carcinoma. When synthesized in insect cells using the baculovirus expression system, the E1 and E2 proteins interacted efficiently at 4 degree. A monoclonal antibody recognizing E1 amino acids 584--600 inhibited the binding of E2 and vice versa, indicating that these amino acids are involved in E2 binding. To confirm this result, a mutational analysis of E1 was performed. The E2 binding activity of E1 deletion and point mutant proteins was assayed using glutathione S-transferase E1 fusion proteins and in vitro translated proteins. At 4 degree, the C-terminal portion of E1 including amino acids 312--644 was sufficient for E2 binding. Introduction of C-terminal deletions or a point mutation at position 586 (Pro --> Glu) resulted in the loss of the E2 binding activity. A second more N-terminally located binding domain (E1 amino acids 312--450) became active when the assays were performed at 22 degrees. The monoclonal antibody still inhibited E2 binding at this temperature, indicating that both E2 binding domains are engaged in the context of the full-length protein."

**PMID**:14985338 **RELATION**:6804\_9751 **FREQ**:0

**Phosphorylation of syntaphilin by cAMP-dependent protein kinase modulates its interaction with syntaxin-1 and annuls its inhibitory effect on vesicle exocytosis.**

We think the difficulty in identifying this relation could be because the information is in multiple sentences.

**Abstract**

cAMP-dependent protein kinase (PKA) can modulate synaptic transmission by acting directly on the neurotransmitter secretory machinery. Here, we identify one possible target: syntaphilin, which was identified as a molecular clamp that controls free syntaxin-1 and dynamin-1 availability and thereby regulates synaptic vesicle exocytosis and endocytosis. Deletion mutation and site-directed mutagenesis experiments pinpoint dominant PKA phosphorylation sites to serines 43 and 56. PKA phosphorylation of syntaphilin significantly decreases its binding to syntaxin-1A in vitro. A syntaphilin mutation of serine 43 to aspartic acid (S43D) shows similar effects on binding. To characterize in vivo phosphorylation events, we generated antisera against a peptide of syntaphilin containing a phosphorylated serine 43. Treatment of rat brain synaptosomes or syntaphilin-transfected HEK 293 cells with the cAMP analogue BIMPS induces in vivo phosphorylation of syntaphilin and inhibits its interaction with syntaxin-1 in neurons. To determine whether PKA phosphorylation of syntaphilin is involved in the regulation of Ca(2+)-dependent exocytosis, we investigated the effect of overexpression of syntaphilin and its S43D mutant on the regulated secretion of human growth hormone from PC12 cells. Although expression of wild type syntaphilin in PC12 cells exhibits significant reduction in high K(+)-induced human growth hormone release, the S43D mutant fails to inhibit exocytosis. Our data predict that syntaphilin could be a highly regulated molecule and that PKA phosphorylation could act as an ""off"" switch for syntaphilin, thus blocking its inhibitory function via the cAMP-dependent signal transduction pathway."

**PMID**:15769741 **RELATION**:285\_285 **FREQ**:0

**Oligomerization and multimerization are critical for angiopoietin-1 to bind and phosphorylate Tie2.**

In this article, some teams correctly identified the relation Ang1-Tie2, however they miss the fact that Ang2 does not interact with Tie2 when mutated, instead it interacts with itself.

**Abstract**

Angiopoietin-1 (Ang1) is an essential molecule for blood vessel formation; however, little is known about the structure-function relationships of Ang1 with its receptor, Tie2 (tyrosine kinase with immunoglobulin and epidermal growth factor homology domain-2'). In this study, we generated several Ang1 and angiopoietin-2 (Ang2) variants to define the role of the superclustering and oligomerization domains of the Ang1 protein. Then we analyzed the molecular structure of the variants with SDS-PAGE and rotary metal-shadowing transmission electron microscopy (RMSTEM) and determined the effects of these variants on the binding and activation of Tie2. Ang1 exists as heterogeneous multimers with basic trimeric, tetrameric, and pentameric oligomers, whereas Ang2 exists as trimeric, tetrameric, and pentameric oligomers. The variant Ang1C265S, consisting of trimers, tetramers, and pentamers without multimeric forms of Ang1, yielded less Tie2 activation than did Ang1, whereas monomeric Ang1 (Ang1/FD), dimeric Ang1 variants (Ang1D2, and Ang1D3), and dimeric and trimeric Ang1 variant (Ang1D1) dramatically lost their ability to bind and activate Tie2. An Ang1 protein in which two cysteines (amino acids 41 and 54) were replaced with serines (Ang1C41S/C54S) formed mostly dimers and trimers that were not able to bind and activate Tie2. In addition, improper creation of a new cysteine in Ang2 (Ang2S263C) dramatically induced Ang2 aggregation without activating Tie2. In conclusion, proper oligomerization of Ang1 having at least four subunits by the intermolecular disulfide linkage involving cysteines 41 and 54 is critical for Tie2 binding and activation. Thus, our data shed a light on the structure-function relationships of Ang1 with Tie2."

**PMID**:9099695 **RELATION**:495516\_495516 **FREQ**:0

**Identification of the autophosphorylation sites of the Xenopus laevis Pim-1 proto-oncogene-encoded protein kinase.**

All teams missed the self-interaction described in this sentence.

**Abstract**

Pim-1 is an oncogene-encoded serine/threonine kinase expressed primarily in cells of the hematopoietic and germ line lineages. Previously identified only in mammals, pim-1 cDNA was cloned and sequenced from the African clawed frog Xenopus laevis. The coding region of Xenopus pim-1 encoded a protein of 324 residues, which exhibited 64% amino acid identity with the full-length human cognate. Xenopus Pim-1 was expressed in bacteria as a glutathione S-transferase (GST) fusion protein and in COS cells. Phosphoamino acid analysis revealed that recombinant Pim-1 autophosphorylated on serine and threonine and to a more limited extent on tyrosine. Electrospray ionization mass spectroscopy was undertaken to locate these phosphorylation sites, and the primary autophosphorylation site of GST-Pim-1 was identified as Ser-190 with Thr-205 and Ser-4 being minor sites. Ser-190, which immediately follows the high conserved Asp-Phe-Gly motif in catalytic subdomain VII, is also featured in more than 20 other protein kinases. To evaluate the importance of the Ser-190 site on the phosphotransferase activity of Pim-1, Ser-190 was mutated to either alanine or glutamic acid, and the constructs were expressed in bacteria as GST fusion proteins and in COS cells. These mutants confirmed that Ser-190 is a major autophosphorylation site of Pim-1 and indicated that phosphorylation of Pim-1 on the Ser-190 residue may serve to activate this kinase."

**PMID**: 9786907 **RELATION**: 1030\_1030 **FREQ**:0

**The open reading frame III product of cauliflower mosaic virus forms a tetramer through a N-terminal coiled-coil.**

We think the difficulty in identifying this relation could be because the information is in multiple sentences. First, the self-interaction is described, then the effect of mutation. When the effect of the mutation is described the protein is referred to as “the tetramer”.

ABSTRACT:

The open reading frame III product of cauliflower mosaic virus is a protein of 15 kDa (p15) that is essential for the virus life cycle. It was shown that the 34 N-terminal amino acids are sufficient to support protein-protein interaction with the full-length p15 in the yeast two-hybrid system. A corresponding peptide was synthesized and a recombinant p15 was expressed in Escherichia coli and purified. Circular dichroism spectroscopy showed that the peptide and the full-length protein can assume an alpha-helical conformation. Analytical centrifugation allowed to determine that p15 assembles as a rod-shaped tetramer. Oxidative cross-linking of N-terminal cysteines of the peptide generated specific covalent oligomers, indicating that the N terminus of p15 is a coiled-coil that assembles as a parallel tetramer. Mutation of Lys22 into Asp destabilized the tetramer and put forward the presence of a salt bridge between Lys22 and Asp24 in a model building of the stalk. These results suggest a model in which the stalk segment of p15 is located at its N terminus, followed by a hinge that provides the space for presenting the C terminus for interactions with nucleic acids and/or proteins.