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PROTEOMICS AND GENOMICS RESEARCH
ISSN NO: 2326-0793

Research Article
DOI: 10.14302/issn.2326-0793.jpgr-13-355

The Role of FIP-2 (optineurin) in Regulation of the Chemokines and Kinases

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ABSTRACT

FIP-2 is a multifunctional protein which is involved in various cellular processes. Using different approaches we investigated its regulatory activity. The microarray analysis has shown that FIP-2 substantially altered the expression of 75 genes (35+/40-) from different functional groups with maximal presentation in "Signal transduction" and "Transcription regulation". Real time RT-PCR indicated significant elevation in the transcription of chemokines, particularly IL-8 (CXCL8). Production of IL-8 in HEK293 cells dramatically increased with FIP-2 overexpression. We also demonstrated that FIP-2 induced activation of IL-8 promoter activity through NF-kB binding site. Additionally, we showed that FIP-2 could interact with PAK3 and increase its kinase activity. Overall, we demonstrated the role of FIP-2 in the regulation of chemokines (IL-8, MPIF-1, MCP-1) and kinases (PAK3, ALK).

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Running title: Regulatory role of FIP-2 (optineurin)

Key words: FIP-2 (optineurin), microarray, gene expression, regulation, interleukin-8, p21-activated kinase 3

Received : Nov 27, 2013; **Accepted :** Jan 04, 2014; **Published :** Jan 30, 2014;

Introduction

FIP-2 (**F**ourteen point seven (14.7K) **I**nteraction **P**rotein-**2**) was originally detected during a yeast two-hybrid screening using an adenovirus early E3-coded protein, 14.7K, as bait [1]. A few years later another protein named NEMO-related protein (NRP) was identified as a protein with partial homology to the well-known component of IKK complex, NEMO/IKK-gamma/ FIP-3 [2]. At that time the data about the transcription factor IIIA-interacting protein also appeared [3]. Finally, a protein named optineurin (OPTN) was detected during genetic analysis of individuals with adult-onset primary open-angle glaucoma [4]. All these proteins have similar sequences and contain 577 amino acids (about 66 kDa) with high levels of Glu (15.8%), Leu (11.8%), Lys (9.4%), and Ser (8.0%). FIP-2 has a few domains such as coiled-coil, leucine zipper, ubiquitin-binding, and zinc finger [1, 4]. FIP-2 is thought to be a multifunctional protein that participates in the development of primary open-angle glaucoma, amyotrophic lateral sclerosis, and Paget's disease of bone [5, 6]. It is still not clear how gain or loss of functions of this protein leads to these and probably other neurodegenerative pathologies. It has been shown that FIP-2 interacts with several proteins such as huntingtin, Rab 8, myosin VI, transferring receptor, TANK-binding kinase 1, serine/ threonine kinase receptor-interacting protein 1, metabotropic glutamate receptors, and transcription factor IIIA. FIP-2 is involved in various cellular functions, including cellular morphogenesis, membrane and vesicular trafficking, transcription activation, maintenance of the Golgi apparatus, signaling, and exocytosis [6]. Our current work demonstrates that FIP-2 regulates chemokines (IL-8, MPIF-1, MCP-1) and kinases (PAK3, ALK). IL-8 and PAK3 could be two important proteins which participate in neuroinflammation/neurodegeneration. Interleukin 8 (IL -8) is a proinflammatory CXC chemokine associated primarily with neutrophil and other granulocyte chemotaxis. The induction of IL-8 signaling activates multiple signaling pathways such as PI3-K and MAPK, which results in activation of numerous transcription factors (NF-kB, AP-1, STAT3 etc.) and expression of proteins involved in inflammation, angiogenesis, cell survival and proliferation, invasion and tumorigenesis [7]. p21-Activated Kinase 3 (PAK3) is a member of the PAK family of serine/threonine kinases, which are involved in the regulation of gene transcription, signal transduction, survival, cytoskeletal reorganization, cell morphology and mobility [8]. Interestingly, PAK3 is predominantly expressed in the brain and is important for embryonic brain development. A direct connection between PAK3 and X-link mental retardation has been shown [9].

Materials and Methods

Cell Lines and Plasmids

HEK293 (Human embryonic kidney cells) and U373 (Human astrocytoma cells) were obtained from American Type Culture Collection (ATCC) and grown in DMEM or a-MEM media accordingly, supplemented with 10% fetal calf serum, penicillin (50 units/ml) and streptomycin (50 µg/ml). pcDNA-T7-FIP-2 expression vector was constructed by cloning the FIP-2 coding region into T7-pcDNA3.1 as previously described (1, 10). IL-8/LUC reporter plasmid was a generous gift from Dr. Antonella Casola (Division of Child Health Research Center, Galveston, Texas) and was previously described in publications from this laboratory [11, 12]. Plasmid pCH110, which expresses b-galactosidase under the control of the SV40 promoter was received from Amersham Biosciences and was used for the normalization of the luciferase reporter assay. Plasmid pGreen-Lantern-1 (pGL), which expresses the Green Fluorescent Protein under the control of the CMV promoter was purchased from Life Technologies and was used to check efficiency of the transfection.

Microarray Analysis

Total RNA (1µg) was isolated from FIP-2 transfected and mock transfected cells using RNAqueous kit and mRNA was further purified with Poly(A)Purist kit (Ambion). Purified RNA was converted to double-stranded cDNA with SuperScript kit (Invitrogen) using oligo-dT primers containing T7 RNA polymerase promoter (Genset). Biotinylated cRNA was prepared from cDNA by *in vitro* transcription with the T7 RNA polymerase (ENZO). The labeled cRNA was fragmented by incubation at 94°C for 35 min. Hybridization, washing and staining were performed according to the Affymetrix technical manual using Affymetrix "human" chip (U95Av2). The chip was stained with streptavidin-

phycoerythrin (Molecular Probes) and scanned with Hewlett-Packard Gene Array Scanner in the AECOM Micoarray facility. Data analysis was performed using Affymetrix GeneChip Analysis Suite software. All data were quantile normalized and the gene list was created with a minimum fold-change of 2. These genes were separated into functional groups according to Gene Ontology (http://www.geneontology.org).

RT-PCR and TaqMan Real-Time RT-PCR (Q - RT-PCR)

Total RNA was purified with a High Pure RNA Isolation Kit (Roche). Single-strand cDNA was synthesized with SuperScript II DNA polymerase according to the manufacturer's protocol (Invitrogen). Primers and TaqMan probes were chosen with Primer Express (version 2.0) software (Applied Biosystems) or used as pre-developed TaqMan Assay Reagents (PDAR) from Applied Biosystems. GAPDH probes from Applied Biosystems were used for normalization. Q – RT-PCR was performed using ABI Prism 7900HT (Applied Biosystems). Quantification of data was performed according to the Applied Biosystems recommendations based on the Delta-Delta Ct method.

Enzyme-Linked Immunosorbent Assay (ELISA)

Quantikine kit (R#D Systems, Minneapolis, MN) for IL-8 was used for the detection of secreted chemokine. The strips with adsorbed specific antibody were incubated with the 50ul prediluted cell culture supernatants and 100ul of the same antibody conjugated with horseradish peroxidase (HRP). After 3.0 h incubation at room temperature the strips were washed and incubated with a substrate in the dark until color appeared. The reaction was stopped with 2N sulfuric acid and readout was performed at 450 nm (substraction at 562 nm) using an ELISA reader (Molecular Dynamics). The amount of IL-8 was determined according to a standard curve and calculated with the GraphPad Prism 5.0 software.

Co-immunoprecipitation (Co-IP) and Western blotting (WB)

The cells were lysed in PBS buffer containing 1% NP-40, 0.5% DOC, and protease inhibitors (Roche). Lysates were precleared and incubated with a specific antibody (anti-FIP-2 /home-made rabbit polyclonal antibody/; anti -PAK3 /rabbit polyclonal antibody from Pierce/) and protein A/G-agarose (Santa Cruz Biotechnology) for 1 h at 4°C each. The agarose beads were then washed 3-4 times with lysis buffer, and proteins were eluted by boiling in Laemmli buffer and separated by SDS-PAGE on a 10% polyacrylamide gel. Following electrophoresis, the proteins were transferred to nitrocellulose and detected with mouse anti-T7 antibody (Novagen) and anti-mouse IgG conjugated with HRP (Amersham Biosciences). The signals were developed using enhanced chemiluminescence (NEN).

In vitro IP-Kinase Assay

IP-kinase assay was performed as previously described [13]. Briefly, PAK3 was immunoprecipitated with anti-PAK3 antibody (Pierce) as described above and agarose beads were incubated in the kinase buffer (20mM HEPES, pH 7.5; 20mM b-glycerophosphate, 10mM $MgCl_2$, 10mM Na_3VO_4, 2mM DTT, and 1x complete protease inhibitor mixture (Roche)) with 2mg MBP, 20mM ATP and 2mCi [g-^{32}P]ATP for 20 min at 30°C. The reaction was stopped by boiling in Laemmli buffer and SDS-PAGE was performed using 10% SDS-polyacrylamide gel.

Luciferase Reporter Assay

HEK293 cells in 6-well plates were transfected using Lipofectamine (Invitrogen) with IL-8/LUC reporter plasmid (0.5µg), pcDNA-T7-FIP-2 (0.5µg), pCH110 (0.2µg), pGL(0.1µg), and pcDNA 3.1 to make DNA concentration equal. After overnight incubation the cells were lysed and luciferase activity was measured by a luciferase assay kit (Roche). Data were normalized according to the b-galactosidase activity.

Immunofluorescence

Immunofluorescence was performed as described earlier (10). Briefly, HEK293 cells grown on the chamber slides (Nunc) were transfected (or not /Control/) with pcDNA-T7-FIP-2 by Lipofectamine (Life Technologies, Inc.) according to manufacturer's protocol. After 24 h, the cells were fixed, permeabilized and incubated with mouse anti-T7 antibody (1 h at room temperature), followed by anti-mouse IgG conjugated with FITC (1 h at room temperature). Analysis was performed using an inverted microscope with an epi-fluorescent attachment.

Statistical Analysis

For multiple comparisons, one-way ANOVA with Bonferoni post test was performed. For comparison of the two groups, Student's unpaired t test was used. p value <0.05 was considered significant. All statistics were run using the GraphPad Prism 5.0 software.

Results:

Microarray Profile Resulting From FIP-2 OverExpression

HEK293 cells were transfected with the pcDNA 3.1 plasmid containing the Long splice variant of FIP-2 with T7-tag. The expression of FIP-2 was confirmed by immunofluorescence (Fig. 1A) and Western blotting (Fig. 1B). Transfected FIP-2 was diffusely distributed in the cytoplasm, although the endogenous protein is mostly associated with the Golgi network [not shown and Ref. 2, 14].

Biotin-labeled cRNAs, which were in vitro transcribed from the mRNAs isolated from FIP-2 transfected (FIP-2 overexpressed) cells and mock transfected cells, were hybridized with a microarray representing over 12,000 transcripts. The microarray data were filtered and a 2 fold cut-off was applied in order to exclude high variability and reach statistical significance. Table 1 represents a list of genes that are up- and down-regulated by more than 2 fold by FIP-2. These genes were separated into functional groups using Gene Ontology (Fig. 2). The most representative groups are: "Signal transduction" (18 members), "Transcription regulation" (9 members), "Oncogenesis" (7 members), and "Anti-Pathogen response" (7 members). Among the most changed genes were "Small inducible cytokine subfamily A (CC)" (172 fold up), "Complement factor H" (43 fold up), "Nedd-4 like ubiquitin-protein ligase WW P2 (9 fold up), "Rod photoreceptor CNG-channel beta subunit (RCNC2)" (14 fold down), "Myc-associated Zinc finger protein (10 fold down), and "Munc 13" (10 fold down) (Table 1). In total, the microarray analysis has shown that FIP-2 substantially altered the expression of the 75 genes.

Validation of Gene Expression

The genes with the highest differences between FIP-2 overexpressed and control (mock transfected) cells were validated by RT-PCR and Q - RT-PCR. RT-PCR data correlated with the microarray results (see Table 1 and Fig.3). The representative example of RT-PCR is shown in Fig. 3A. At the top of the FIP-2 up-regulated genes was "Small inducible cytokine subfamily A". This is a chemokine gene cluster on human chromosome 17q 11.2 containing five CC chemokine genes: MPIF-1 (CCL23), HCC-2 (MIP-1, CCL15), HCC-1 (CCL14), LEC (CCL16, HCC-4), and RANTES (CCL5) [15]. The preliminary analysis showed that only MPIF-1 was significantly up-regulated with FIP-2 overexpression (Fig. 3A and not shown). Next, we performed Q - RT-PCR to examine the changes of these proteins in FIP-2 transfected cells, but besides MPIF-1 we also included other chemokines, particularly MCP-1 (CCL2) and IL-8 (CXCL8). Although MPIF-1 and MCP-1 were significantly up-regulated in FIP-2 transfected cells, the most dramatic change was observed for IL-8 (Fig. 3B). Then these data were analyzed in more details.

FIP-2 Regulates the Expression of Interleukin 8

HEK293 cells as and human astrocytoma (U373) cells transfected with FIP-2 were tested for the production of IL-8 by ELISA. In both cell lines we determined high IL-8 in comparison with the low basal level in the control mock transfected cells (Fig. 4A). This result was confirmed in the dose-response experiments. When using 1µg of DNA (FIP-2 plasmid) per 10^6 cells we observed that the production of IL-8 increased by 25-30 fold. The cells treated with TNFα were used as a positive control (Fig. 4B).

In order to understand the mechanism of FIP-2 activity, we tested the ability of FIP-2 to change the IL-8 expression by binding with its promoter. We used the luciferase reporter assay and IL-8/LUC reporter plasmids: a) -1500/+44 IL-8/LUC plasmid, which contains the first 1500 bases of the IL-8 promoter and b) -162/+44 IL-8/LUC plasmid with minimal IL-8 promoter. This deletion does not affect the inducibility of the promoter [11, 12]. FIP-2 induced IL-8 promoter activity in both cases, which means that the minimal sequence of the promoter was enough for the FIP-2 binding (Fig. 4C). Actinomycin D, a RNA synthesis inhibitor, completely blocked IL-8 gene transcription, which confirmed the specificity of this reaction (Fig. 4C). To define the region of the IL-8 promoter involved in the regulation of gene expression with FIP-2 overexpression, HEK293 cells were transfected with -162/+44 IL-8/LUC plasmid with deletions in binding sites such as NF-kB and AP-1. As shown in Fig. 4D, the mutation of the NF-

Table 1. Up- and down-regulated genes at FIP-2 overexpression

Up-regulated genes		
Name	**Accession No.**	**Fold Change**
Small inducible cytokine subfamily A	AF088219	171.6
Complement factor H	X07523	43.2
Nedd-4 like ubiquitin-protein ligase WWP2	U96114	8.7
Transglutaminase	M55153	6.1
Hepatic leukemia factor	M95585	4.9
Galectin-4	AB006781	4.8
ELAV-like neuronal protein-2 Hel-N2	U29943	4.7
Anaplastic lymphoma kinase	U62540	4.4
GAGE-4 protein	U19145	4.4
ERBB-3 receptor protein-tyrosine kinase precursor	HO6628	4.3
Type VI collagen alpha-2 chain precursor	M20777	4.1
Filament protein CP49 (LIFL-L)	U48224	4.0
EMX 2	X68880	3.8
RAD 51D	AF034956	3.3
Prot-oncogene (BMI-1) L13689	L13689	3.2
Gamma-actin	D00654	3.2
TROP-2 (tumor-associated calcium signal transducer 2)	X77753	3.1
Myelin basic protein	M13577	3.1
Alu-binding protein with Zinc finger domain	X83877	3.1
Killer cell lectin-like receptor subfamily C	AJ001685	2.9
MOBP (myelin-associated oligodendrocytic basic protein	D28113	2.9
Transferrin	S95936	2.8
Glycoprotein 6-alpha-L-fucosyltransferase	Y17979	2.6
WW-domain-containing protein WWP3	U96115	2.6
Putative GTP-binding protein similar to RAY/RABIC	AL022729	2.5
DNA fragmentation factor-45	U91985	2.4
36 kda FK 506 binding protein	AF038847	2.4
p21-activated kinase 3	AF068864	2.4
Alpha-1 type XVI collagen (COL 16A1)	M92642	2.3
Glycoprotein (transmembrane)	NMB X76534	2.2
Gap junction protein (Connexin 32)	X04325	2.2
60 kDa ribonucleoprotein autoantigen SS-A/Ro	J04137	2.2
Bcl-2	M14745	2.1
Heat shok protein HSPA2	L26336	2.1
Small GTP-binding protein Rab 27b	U57093	2.1

Down-regulated genes		
Name	**Accession No.**	**Fold Change**
CDP-diacylglycerol synthase 1	U65887	2.0
DNA helicase Q1-like (RECQL)	L36140	2.0
Ribonuclease P protein subunit p14	AF001175	2.0
Proteosome subunit p42	D78275	2.0
Corproporphyrinogen oxodase	D16611	2.0
Insulin receptor substrate 1	S62539	2.1
X-like 1 protein	AJ005821	2.2
Thyroid hormone receptor coactivatibg protein	X87613	2.3
HIV tata element modulatory factor	L01042	2.3
Metastasis-associated MTA 1	U35113	2.4
Zink finger protein (ZnF20)	AF011573	2.4
Cell membrane glycoprotein 110 kDa	D64154	2.5
Integrin, alpha subunit	X68742	2.7
XP-C repair complementing protein (p125)	D21089	2.8
Leukocyte immunoglobulin-like receptor 8	AF025534	3.0
Paired box gene 1	AL035562	3.0
Cytochrome P450	J04813	3.1
Purinoreceptor P2X3	Y07683	3.3
IL-4	X81851	3.4
Aplha 2 Actinin	M86406	3.6
Proteolipid protein 2	U93305	3.8
Adducin 1	L07261	4.2
PEST phosphatase interacting protein 1	U94778	4.6
Tyrosine phosphatase receptor C	Y00638	4.8
TGF-beta type III receptor	L07594	5.2
Transmembrane receptor IL-1R	U43672	5.2
Ste 20-like kinase	X99325	5.4
DNA-binding protein GLI3	M57609	5.5
LDL receptor related protein 105	AB009462	5.5
CDC-like kinase 3 isoform HCLK3/152	L29217	5.5
CRK-II (avian sarcoma virus CT10 oncogene homolog)	D10656	6.6
Transmembrane protein WFS1 (Wolframin)	AF084481	7.1
Stimulator of Fe transport	AF020761	7.4
MB-1	U05259	7.6
PTB-associated splicing factor	W27050	8.3
Munc 13	AF020202	9.5
Myc-associated Zinc finger protein	D85131	10.1
Atrial Natriuretic peptide ANP, Prepronatriodilatin	AL021155	10.9
Guanylate binding protein isoform II	M55543	11.3
Rod photoreceptor CNG-channel beta subunit	AF042498	14.0

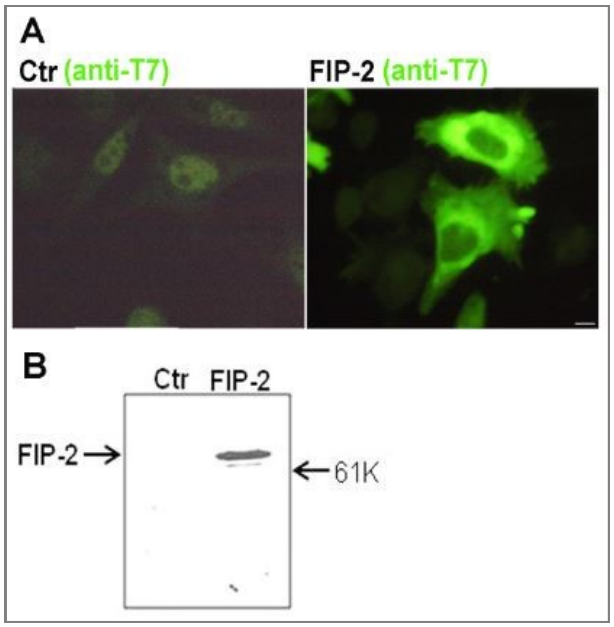

Figure 1. FIP-2 overexpression in HEK293 cells.

HEK293 cells were transfected with T7-FIP-2 plasmid or empty vector (Control/Ctr) and tested by immunofluorescence (**A**) and Western blotting (**B**) with anti-T7 antibody as described in Materials and Methods. Scale bar = 200 mM.

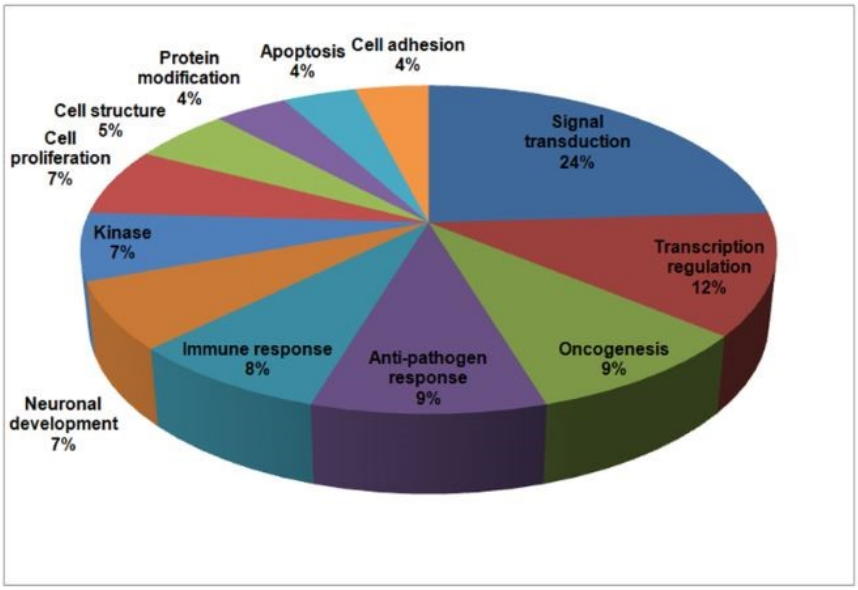

Figure 2. Functional groups of genes which are up- and down-regulated by FIP-2.

The genes were separated into groups using Gene Ontology and are presented as percentages for each functional group.

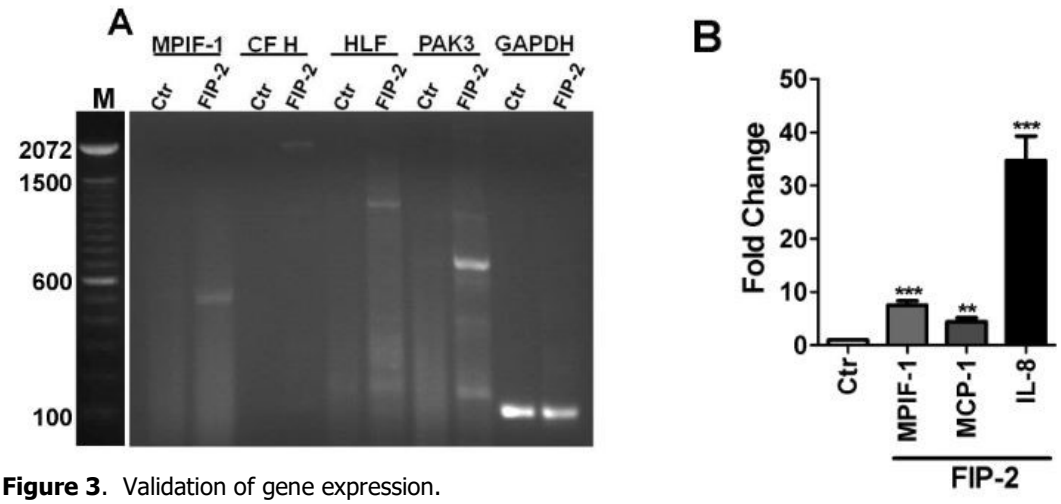

Figure 3. Validation of gene expression.

Total RNA from the mock (Ctr) and FIP-2 transfected cells was tested by RT-PCR (**A**) and Q – RT-PCR (**B**) using specific primers as described in Materials and Methods. GAPDH was used as a control housekeeping gene. M – Markers (100bp DNA Ladder from Life Technologies/Invitrogen). MPIF-1 - Myeloid progenitor inhibitory factor 1, CF H – Complement Factor H, HLF – Hepatic Leukemia Factor, PAK3 - p21 protein (Cdc42/Rac)-activated kinase 3, GAPDH - Glyceraldehyde 3-phosphate dehydrogenase, MCP-1 - monocyte chemotactic protein-1, IL-8 – Interleukin-8 (CXCL8, chemokine (C-X-C motif) ligand 8).

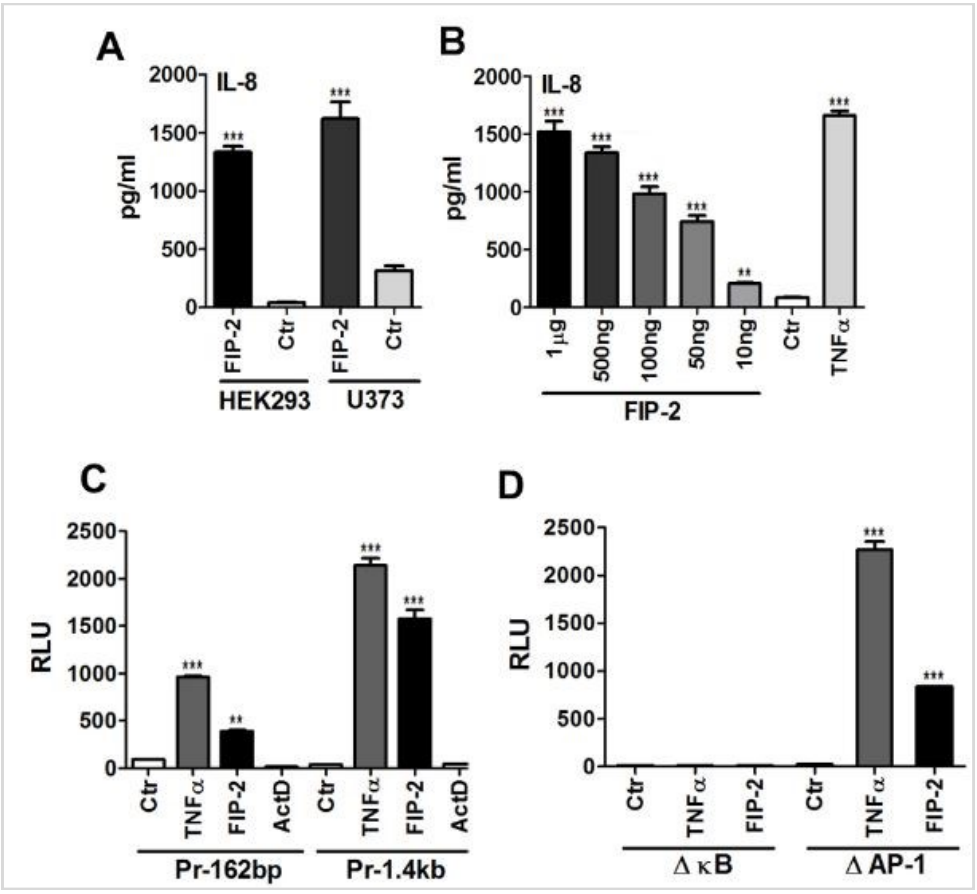

Figure 4. FIP-2 regulates the expression of IL-8.

(**A, B**) IL-8 production at the FIP-2 overexpression. HEK293 and U373 cells were transfected with FIP-2 (0.5 µg) for 24h and the protein production was estimated by ELISA as described in Materials and Methods.
A: transfection of the HEK293 and U373 cells. **Ctr** - transfection with empty vector;
B: HEK293 cells were transfected with different doses of FIP-2 as indicated and after 18 h incubation the IL-8 was determined in supernatants by ELISA. TNFα (stimulation during 18 h with 10 ng/ml) was used as a positive control. The representative data performed in triplicate is shown.
(**C, D**) Transcriptional induction of IL-8 by FIP-2.
C: HEK293 cells were transiently transfected with minimal IL-8/LUC promoter [-162/+44] (Pr-162bp) alone, with TNFα stimulation, or FIP -2 overexpression, or with FIP-2 in the presence of actinomycin D (ActD) and full IL-8/LUC promoter [-1.4/+44] (Pr-1.4kb) alone, with TNF α stimulation, or FIP-2 overexpression, or with FIP-2 in the presence of actinomycin D (ActD).
D: HEK293 cells were transiently transfected with site-mutated plasmids of the -162/+44 IL-8/LUC promoter: NF-kB or AP-1 and stimulated with TNFα or with FIP-2. The representative data performed in triplicate is shown. RLU – relative luciferase units.

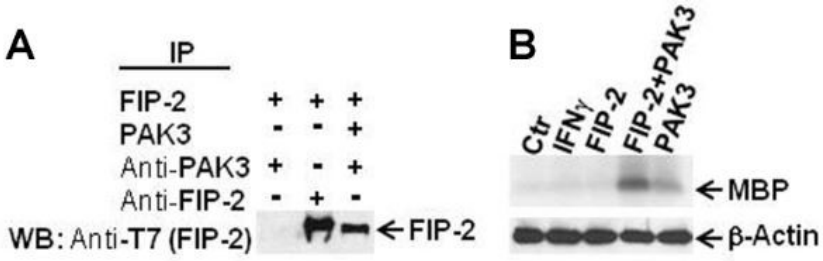

Figure 5. FIP-2 regulates the PAK3 activity.

(**A**) Co-immunoprecipitation. HEK293 cells were transfected with pcDNA3.1 plasmid containing FIP-2 or PAK3, or both and precipitated with anti-FIP-2 or anti-PAK3 antibody followed Western blotting with anti-T7 antibody.
(**B**) In vitro kinase assay. Kinase assay was performed with the cell lysates from HEK293 cells alone (Ctr), cells treated with IFNγ, or transfected with FIP-2 or PAK3 or together. β-Actin was used as loading control.

The results of microarray analysis (see Table 1) and RT -PCR (Fig. 3A) indicated that the expression of PAK3 was significantly increased with FIP-2 overexpression. To

study the relationship between these two proteins, we first performed a co-immunoprecipitation assay. We transfected HEK293 cells with FIP-2 (tagged with T7) and PAK3 separately and together and immunoprecipitated with antibody against the FIP-2 and PAK3, followed by Western blotting using the anti-T7 (FIP-2) antibody. Anti-PAK3 antibody did not precipitate FIP-2 alone but in the presence of PAK3 it precipitated FIP-2, which was then detected by Western blotting (Fig. 5A). This result indicates the presence of protein-protein interaction between FIP-2 and PAK3.

Then we performed an *in vitro* kinase assay using MBP as a substrate to test the ability of FIP-2 to change PAK3 kinase activity. We demonstrated that FIP-2 could increase the PAK3 kinase activity by 3-4 folds, in comparison with PAK3 alone. No kinase activity was associated with FIP-2 alone (Fig. 5B). Thus, FIP-2 could interact with PAK3 and increase its kinase activity.

Discussion

FIP-2 is a multifunctional protein that is involved in many biological processes in the cell. Here we showed that FIP-2 (OPTN) up-regulates the transcription of chemokines, particularly IL-8 (CXCL8), through the NF-kB binding site of IL-8 promoter, although other mechanisms (like participation of the intermediate proteins/transcription factors) could not be ruled out. Data about the mutated optineurin in individuals with glaucoma suggested that this protein has neuroprotective functions in the optic nerve and may be related to the TNFα signaling pathway and oxidative stress [4, 16, 17]. It is interesting that while there was an increase in the expression of the proinflammatory chemokine IL-8, FIP-2 decreased the expression of the anti-inflammatory cytokine IL-4 (see Table 1). Thus, the overexpression of FIP-2 could induce the neuroinflammation followed by neurodegeneration which was observed in glaucoma (degeneration of optic nerve) and amyotrophic lateral sclerosis (death of motor neurons).

Microarray analysis showed that FIP-2 could change the expression of a number of kinases, such as anaplastic lymphoma kinase, p21-activated kinase 3 (PAK3), CDC-like kinase 3, and Ste 20-like kinase (see Table 1). Our attention was drawn to PAK3 because of the RT-PCR results (Fig. 3A). We demonstrated that FIP-2 interacts with PAK3 and increases its kinase activity (Fig. 5). Interestingly, PAK3 is implicated in neurodegenerative diseases and specifically involved in mental retardation [9, 18]. We could not exclude the potential role of FIP-2 in this process.

kB site completely abolished the FIP-2 or TNFα-induced promoter activity. In contrast, there was no effect when AP-1 site was deleted (Fig. 4D). This result suggests that NF-kB site is involved in the regulation of IL-8 transcription by FIP-2.

FIP-2 interacts with PAK3 and increases kinase activity

Earlier it was shown that FIP-2 could function in the high molecular weight complex (400-700 kDa) [2, L.T., unpublished]. The components of this complex are not yet identified, but Schwamborn and colleagues [2] demonstrated that it includes two unidentified kinases with molecular weights of 85 and 180 kDa. It is possible that anaplastic lymphoma kinase (ALK) with related molecular weight (176 kDa) could be one of these kinases. Another candidate may be TANK-binding kinase 1 (TBK1) which has a molecular weight of 84 kDa, which also interacts with FIP-2 (OPTN) [19, 20]. This requires further investigation.

In conclusion, we identified the role of FIP-2 (OPTN) in the regulation of chemokines and kinases. This is an important addition to the understanding the mechanism of FIP-2 and its role in the development of neurodegenerative (and possible other) diseases.

Conclusion

Microarray analysis of the FIP-2 overexpressed cells revealed a number of the up- and down-regulated genes. Among them we selected the groups of chemokines and kinases. We demonstrated that FIP-2 significantly increases the expression of FIP-2 through the NF-kB binding site of IL-8 promoter. Additionally, FIP -2 interacts with p21-activated kinase 3 and increases its activity. FIP-2 is a multifunctional protein and a demonstration of its targets will help to determine the most important ones and direct therapy for neurodegenerative and other FIP-2 – related diseases.

Acknowledgement:

We thank Dr. Antonella Casola for the generous gift of IL-8/LUC reporter plasmids and AECOM microarray facility for the help with microarray analysis. This work was supported by grant RO1 CA 72963 (M.S.H, L.T.) and Oncology Research Faculty Development Program (L.T.). We are grateful to Jacqueline S. Coley for critical reading of the manuscript.

Conflict of Interest:

The authors have no competing interests.

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