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Guanine Exchange Factor P92GEF target RhoA and Inhibit Cell Proliferation and Invasion as a Tumor gene*

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ABSTRACT: Dbl family guanine exchange factor (GEFs) is the main regulatory unit for the malignant transformation of Rho family proteins. It plays an important role in the transformation of Rho protein from the inactive GDP form to the active GTP form and participates in the cytoskeleton rearrangement, the cell growth and vitality. This study conducted a preliminary structural and functional study of a typical family member, P92GEF, and discussed the role of the molecule in the development of tumor. The expression of P92GEF in 48 normal tissues of human body was measured by Realtime PCR; GST-pulldown technique was used to detect the GEF activity of P92GEF in vivo; the dual-luciferase reporter gene detection technique was used to test the transcription factor activity of the small downstream molecules, and the high expression of P92GEF was tested by the double staining immunofluorescence method. Effects of high expression of P92GEF on cell proliferation, invasion and tumorigenic ability in vitro were examined using CCK8, Transwell and soft agar clony formation assays. The results of bioinformatics analysis showed that P92GEF contains 841 amino acids, has typical Dbl family molecular domain. The expression of P92GEF in the lung tissue was highest. P92GEF binds to RhoA in vitro and promotes the proliferation and invasion of NIH3T3 cells, and has significant ability to clone in vitro. P92GEF is a typical guanine exchange factor family molecule, which can activate Rho family RhoA and has obvious oncogene characteristics.

Key words: Guanine nucleotide exchange factors; P92GEF; Dbl family; Rho GTPases; Tumor gene

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Rho family GTPase regulates the cell migration, infiltration and transfer by recombination of cytoskeleton, and it is also associated with cell growth and differentiation through regulation and control on the growth cycle of cell [1-3]. It is expressed in a high percentage of several malignant tumors, and there is a close cor re lation between Rho family GTPase and the occurrence, development, incursions and metastasis of tumors[4-7]. Rho GTPase exists in cells usually in an inactive GDP form. It would transform to GTP form by the effect of Guanine Nucleotide Exchange Factors (GEFs)[8,9]. GEFs (Dbl family members) are mainly formed of a DH domain (Dbl Homology Domain) comprising of 200 amino acid and a PH domain (Pleckstrin Homology Domain) comprising of 100 amino acid[10].

The vitro experiment indicates that DH can transform the Rho family proteins from GDP to GTP independently (That means to activate the Rho family protein). The major function of PH is to direct the GEFs to cell membrane area with high level of Rho family protein by associating with the related molecules of cell membrane and the tandem DH-PH domain is the basic function unit of Dbl family members[11].

Dbl family members were considered to play a key role in a large amount of tumorigenesis [12, 13]. The HGP ju st finished con firmed there are nearly 80 members in Dbl family, and new Dbl family oncogenes were found continuously in recent years.

Here we report a GEF family member, P92GEF. It was de scribed by Hisakazu Ogita in 2003 and was called Vsm-RhoGEF[14], which has a high expression in heart and has a specify RhoA activation. There are completely new emphases of our research on P92GEF, to investigate its characteristics as on cogene, and to analysis its effect on tumor signaling pathway by transcription factor activity test. In this article, Alignment found it has high homology with another Dbl family member-ephexin. Re altime PCR analysis showed that the expression of P92GEF is mainly in lungs, and the detection of activity in vitro showed it has GEF activity of RhoA and P92GEF induced stress fiber formation in NIH3T3 fibroblast. Moreover, we've detected the effect of ma lignant biological behavior to NIH3T3 fibroblast by P92GEF, such as colonies formation, migration, proliferation and invasion. The results suggested P92GEF was a typical tumor gene.

1 Materias and methods

1.1 Database search and protein sequence analysis

P92GEF (KIAA0915) complete sequence was scanned by NCBI blastp and contrast with mammalian databases. (EST: AW767483, AW766753, AW211224).

The analysis of structural and functional domain of P92GEF was obtained by SMART [15], and PFAM23.0 (Wellcome Trust Sanger Institute, Canbrige.UK).

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The multiple Sequence alignment between P92GEF and GEFs members was obtained by Software Analysis clustalX 2.0[16], and alignment of aln result was obtained by Software GeneDoc[17]. The cladogram calculated by distance matrix was obtained by tree view（21. Page, R. D. (1996) Comput. Appl. Biosci. 12, 357-358）and MEGA4.1[18].

1.2 Expression plasmids

cDNA clone of P92GEF was obtained as a human expressed sequence tag (EST) clone (AW767483). To perform mammalian cells, P92GEF-fulllenth and P92GEF-DHPH were generated by PCR and subcloned into pDest26-his vector, pRK5-myc vector and pSV vector. RhoA，Cdc42, Rac1 were generated by PCR and ligated into pCMV-HA vector. pGEX-GST-PAK, pGEX-GST-mDia, pCEFL-GST-RhoAV14 expression vector were kindly pro vided by Professor Zheng (Cincinnati Children's hospital Medical Center，Tennessee, USA).

1.3 Materials and Antibodies

Anti-HA antibody was from eBioscience; anti-myc antibody was from Biolegend, anti-GST antibody was from Cell Signaling; Rhodamine-phalloidin was from Sigma-Aldrich (St louis, Mo); Immobilized Glutathione was from Thermo (Thermo Scientific, Rockford); Lipofectamine 2000 reagent was from Invitrogen (Invit rogen，CA); IRDye 800CW Donkey anti-rabbit/mouse IgG (H+L) highly cross adsorbed was from LI-COR IRDye (LI-COR, Lincoln)Dual-Luciferase Reporter Assay Kit was from Promega.

1.4 Production and Purification of GST Fusion Proteins

The binding assay of RhoA, Cdc42 and Rac1 were taken using fusion protein of mDia（GST-RBD）and PAK（GST-PBD）as bait protein, to detect the protein activity of Rho family through GST pull down. GST fusion protein was prepared from *E.coli* BL21, which induced by 0.1 mM IPTG when cell OD reached 0.7~1.0. Rho protein was expressed in 293T cells, which was transfected with relevant Rho plasmid and empty vectors. Cell was collected after 48h culture, and the protein was extracted by RIPA. Rho protein lysis incubated with GST beads binding protein for 3h，washing 3 times with PBS+1% triton, and then washing 3 times with PBS, suspender with SDS buffer and analysised with SDS-page.

1.5 Focus formation analysis

NIH3T3 fibroblast was cultured in vitro to 70% adherent plate; and transfected by P92GEF expression constructs or empty vector with lipofectamine2000; empty plasmid was used as negative control, Dbl expression vector was used as positive control. Cell cultured with DMEM-F12 medium（Invitrogen Company）, 10% fetal serum, 1% penicillin/streptomycin. Stable transfection wit h 0.4 mg/mL G418, culture 22 days. Then, stained with 0.01 g/mL methylene blue and 50% methanol .

1.6 Realtime PCR

Primers were designed by primer 6.0 to specifical amplified P92GEF in human cDNA（Human Major Tissue qPCR Array containing first strand DNA from 48 tissues. Human Rapid-Scan Plate, OriGene Technologies, Inc., Rockville, MD）[Forword primer: 5-AGTCAGCGAGCGGTTTCTA-3; Reverse primer-: 5CGCACATAATCCACATACAC - 3]. Reaction volume was 16.3 μL, contains 600 nmol/L of each primer, 200 nmol/L probe, 0.5 units of Platinum Taq polymerase, 200 μmol/L each of dATP, dCTP, dGTP, dTTP, and 1× PCR buffer. The thermal profile for the PCR was 95℃ for 15 seconds, 57℃ for 30 seconds, and 72℃ for 1 minute, Data obtained after 40 cycles of amplification were analyzed by iQ5 Real-Time PCR Detection System (Bio-Rad) .

1.7 Cell culture, transfections and immunofluorescence microscopy

293T cell cultured with DMEM medium, 10% calf serum, NIH3T3 fibroblast cultured with DMEM-F12 medium, 10% fetal serum, 5% CO_2, 37℃. NIH3T3 fibroblast was stable Transfected by Concentration gradient screening with G418.

8 mm slides were put in 24 well plate after treatment by ly sine, NIH3T3 fibroblast was inoculated, and P92GEF (1 μg) was trans fected. After 48h, washed with PBS and fixed with paraformaldehyde, wall-broken disposed with 1% triton-PBS. Primary antibody incubation with anti-myc, Fluorescein isothio cyanate (FITC)-labelled goat anti-mouse IgG antibody, and of actin filaments with tetramethyl-rhodamine isothiocyanate (TRITC) -conjugated phalloidin, detected by confocal microscopy.

1.8 Cell proliferation Assay

The 96 well plate, with 100 μL medium in each well, was seeded by NIH3T3 fibroblast in a quantity of 3000 cells per well, and cultured at 37℃. Each well was added with 10 μL Cell Counting Kit 8 agent in 0, 1, 2, 3, 4, 5th day, oscillated slightly, blended, and cultured in incubator at 37℃ for 4 hours. Then detect OD450 of cell culture (Biorad Microplate Reader 680). Each experimental group was set triplicate samples, and 3 blank controls.

1.9 Wound-healing assay

The 6 well plate was seeded with 1.5× 10^5 cells, and cultured in bacteriological incubator at 37℃, 5% CO_2 for 24h. Cleaned 1-mm-wide wound area on the bottom of every plate produced by eppendorf pipet, washed the plate and cultured in DMEM-F12 medium with 1% FBS for 48h. Observed the cell migration by inverted microscope and repeated this test three times.

1.10 Invasion assay

BD Matrigel™ Basement Membrane Matrix was diluted with serum free medium in a proportion of 1:2.5 according to the in struction book. Absorbed 35 μL of the solution to spread the well (millipore, Billerica, MA) bottom, placed it for 2h at 37℃. Once set, method of cell trypsinization was taken with 0.25% of pancreatin（digested the cell with 0.25% trypsin）. When observed cells contract and become round, throwed away the excess trypsin, patted the culture flask gently. Added 1% FBS DMEM-F12

medium in culture flask and blended cells. Each well was seeded with 1.5×10^5 cells. There was 1% FBS medium in each well and 800 μL DMEM-F12 with 10 % calf serum in the bottom of well, cultured at 37℃, 5%CO_2 for 48h. Then wiped off bottom gel with cotton swab, HE stained, and counted the number of cells which crossed the well by microscope.

1.11 Soft agar colony formation assay

This experiment was used for verification independent colony formation ability of P92GEF in HEK293 cell. Add the same amount agar at 1.2% (w/v) to 2*DMEM medium with 20% FBS to make media "bottom agar", plated onto 6 well plate. Then sus pended cells in 2*DMEM containing 20%FBS with same amount of agarose solution at 0.65% (w/v) to prepare media "soft agar." plated onto solidified on "bottom agarose" in 6 well plate at a density of 2500 cells per well in triplicate. Cultured cells in 37℃ and replaced the medium twice a week. Counted the colony number by Fluorescence microscopy after cultured 15 days after being stained with 4',6- diamidino- 2-phenylindole.

1.12 Transcription factor activity

Transcription factor activity was tested by Dual-Luciferase Assay Kit (Promega), target gene, downstream micromolecule transcription factor(Firefly luciferase), internal parameter (PRL-TK) were used to transfect 293T, cultured 48h later, lysated with passive lysis buffer, incubating 20 min with gentle wave, then blending with peptide, transferring to 96well plate. Assay transcription fac tor activity by LI-COR synegy system. Detecting transcription factor activity by firefly luciferase, internal parameter (PRL-TK) activity by renilla luciferase activity, using pTA as blank control.

2 Results

2.1 Protein blast analysis and function domain

We've found this GEF molecule by HGP. Protein sequence of P92GEF was analyzed by Blastp, and it was proved to be a typical GEF molecule, located at chromosome: 17; Location: 17p13.1, related with RhoGEF15, encoding a protein with 841 amino acids, molecular weight is about 92KD, and thereby called P92GEF (Fig.1A). The structural and functional domain of protein se quence of P92GEF was analyzed by SMART, PMAF and BlastP, it revealed that the function domains of P92GEF were typi cal DH domain (426~606aa)and PH-like domain (640~751aa)which also identificated as ephexin-PH domain (Fig.1B). Ephex in- PH domain view PSSM is CD01221, which has been well characterized further[19,20]. The result showed that P92GEF was a typical GEF molecule, without other peculiar functional domain.

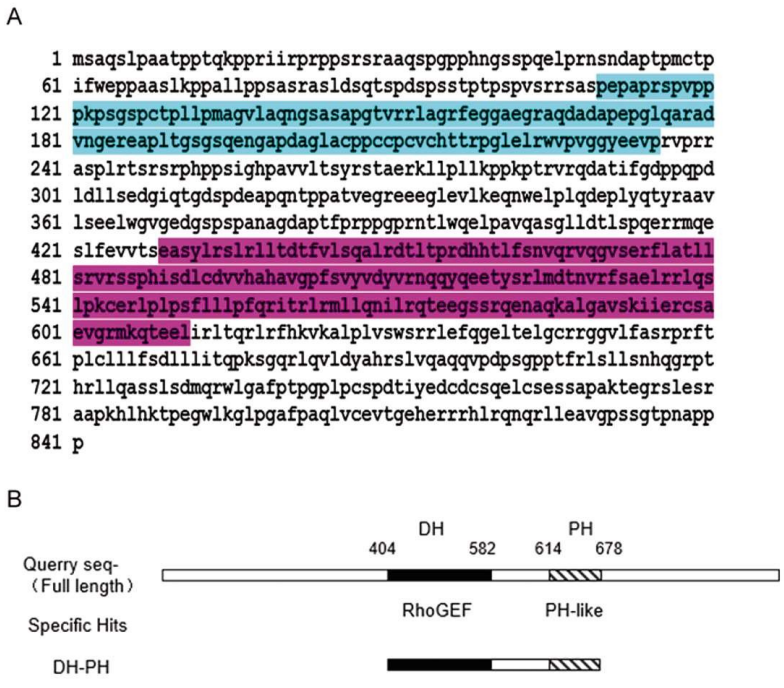
P92GEF sequence alignment was compared with other Dbl family members by Clustal software analysis, and the results was exported by Genedoc (Fig. 1C). Through the evolutionary trace method, homology between P92GEF and most of the Dbl family members is relatively low, but alignment of ephexin revealed a high degree of conservation within the DH-PH domain(Fig.1D).

2.2 The expression of P92GEF in human tissues

The expression of P92GEF was detected in 48 normal human tissues. The realtime PCR was taken using P92GEF primer and human tissue library from OriGene Company, and the result showed Melt Curve with good product specificity. The experiment result showed that there was high level expression in adrenal gland, small intestine, lungs, placenta and vena cava, and highest in lungs. The expression was lower in bone marrow, brain, muscle, testis, thyroid gland, tongue and uvula, and lowest in testis(Fig. 2).

2.3 Binding capacity of P92GEF with Rho family proteins

Fig .3 A. GEF Activity of P92GEF tested by GST-Pull down assay in vivo. To check the GEF activity of P92GEF in vivo, we



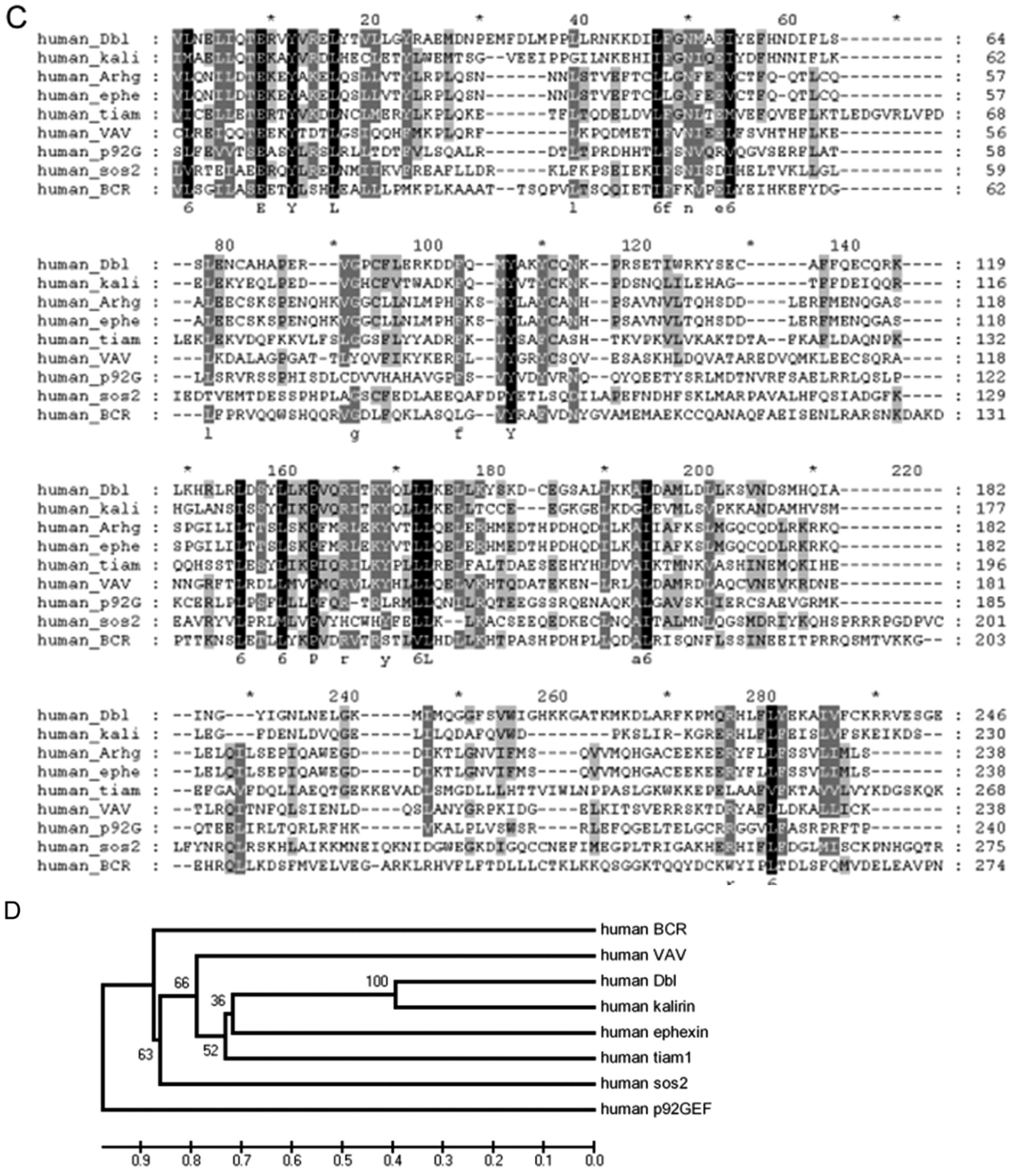


Fig. 1 P92GEF gene sequence analysis

A: P92GEF(KIAA0915) complete sequence was scanned by NCBI blastp and it contained DH and PH domains in residues 426~606 and 640~751 respectively.

B: The function domain:DH domain(Dbl homology domain annotated as Rho GEF) and PH domain((pleckstrin homology domain annotated as ephexin PH domain) was characterised by NCBI Protein Blast and SMART5.0 (SMART5.0 analysis result data was not shown).

C and D: Evolution tree(C) and alignment(D) were taken to compare the sequence homology of P92GEF to other GEFs as known. The Evolution tree was analysised by MEGA4.1 (Molecular evolutionary Genetic Analysis Version 4.1), and digits on cladogram represented the reliability percentage in bootstrap checking. Alignment of aln result was obtained by Software GeneDoc, different gray value residues represented sequence similarities.

used GST beads binding PAK1/mDia to pull down small G protein (RhoA, Cdc42, Rac1)which over expressed in HEK 293 cell, then, tested the result by western blotting. Identified small G pro tein by used of HA-antibody, P92GEF was blotted by Myc- Antibody. All data are representative of at least three independent experiments. B. P92GEF promoted the formation of stress fiber mediated by ac tivating RhoA in 293 cell. After 48h of cell transfection, we carried out immunodetection of tagged protein over expressing by fixing cells on slides, detected the expression of P92GEF by FITC coupling anti-mouse(Left), and detected F-actin in cytoskeleton by rhodamine conjugated phalloidin (Right). This experiment was representative of at least three independent experiments.

To confirm the relationship between P92GEF and Rho family proteins, we applied DH-PH domain to constitute eukaryotic expression vector, and had a further study on the interaction of P92GEF and Ras-Rho family proteins.The ligand-binding assay in

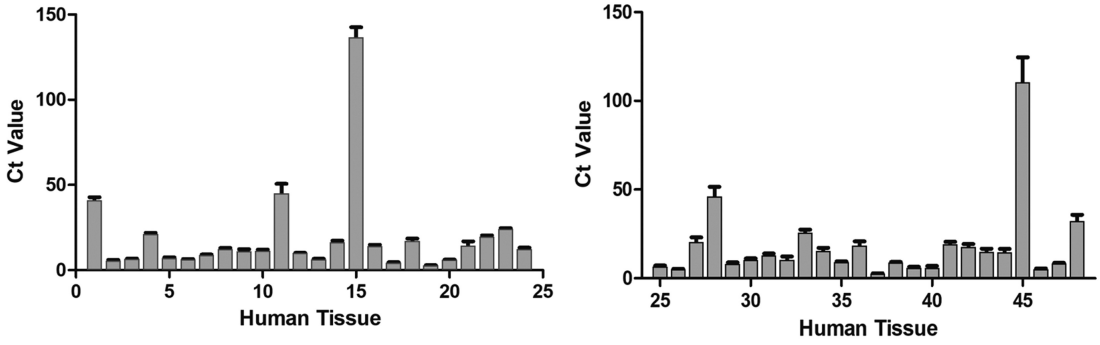


Fig.2 P92GEF Expression in 48 human major tissue

Expression level tested by Real-Time PCR, Normalized relative expression levels among 48 tissue samples.

1.Adrenal Gland 2.Bone Marrow 3.Brain 4.Cervix 5.Colon 6.Descending part of duodenum 7.Epididymis 8.Esophagus 9.Fat 10.Heart 11.Intestine(Small) 12. Intracranial Artery 13.Kidney 14. Liver 15.Lung 16.Lymph Node 17.PBL (plasma blood leucocytes) 18.Mammary gland 19.Muscle 20. Nasal Mucosa 21.Optic Nerve 22.Ovary 23. Oviduct 24. Pancreas 25.Penis 26.Pericardium 27.Pituitary 28.Placenta 29.Prostate 30.Rectum 31.Retina 32. Seminal Vesicles 33. Skin 34.Spinal Cord 35.Spleen 36.Stomach 37.Testis 38.Thymus 39.Thyroid 40.Tongue 41.Tonsil 42.Trachea 43.Urethra 44.Urinary Bladder 45.Uterus 46.Uvula 47.Vagina 48.Cava

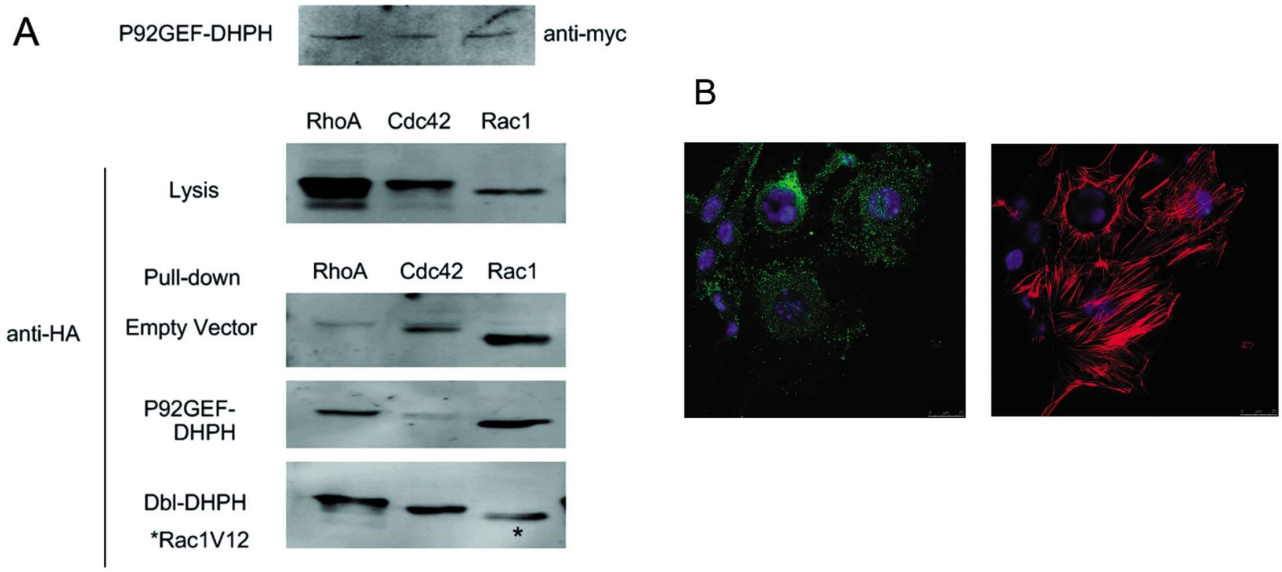


Fig. 3 A.GEF Activity of P92GEF tested by GST-Pull down assay in vivo. To check the GEF activity of P92GEF in vivo, we used GST beads binding PAK1/mDia to pull down small G protein (RhoA, Cdc42, Rac1)which over expressed in HEK 293 cell, then, tested the result by western blotting. Identified small G protein by used of HA-antibody, P92GEF was blotted by Myc- Antibody. All data are representative of at least three independent experiments. B. P92GEF promoted the formation of stress fiber mediated by activating RhoA in 293cell. After 48h of cell transfection, we carried out immunodetection of tagged protein over expressing by fixing cells on slides, detected the expression of P92GEF by FITC coupling anti-mouse(Left), and detected F-actin in cytoskeleton by rhodamine conjugated phalloidin(Right). This experiment was representative of at least three independent experiments.

vitro was applied using GST-pulldown in 293 cells. RhoA/Cdc42/Rac1/Rac1V12 and P92GEF-DHPH/ Dbl-DHPH/ Empty vector were co-transfected, then cell lysis was combined with GTS-mDia and GTS-PAK1, using Dbl as activating control with RhoA and Cdc42 , Rac1 V12 as Rac1 activating control (Fig 3A). The results showed that P92GEF played a role in RhoA activation, and it also played a role in Rac1 activation, which was weaker than RhoA activation.

2.4 The effect of P92GEF to NIH3T3 cytoskeleton

We have found P92GEF could activate RhoA and Rac1 in vi vo through GST pull down experiment. Moreover, We tested whether P92GEF induces the assembly of actin stress fiber, which is a typical consequence of RhoA activation in NIH3T3 fibroblast. The results showed that overexpression of P92GEF induced formation of stress fiber in NIH3T3 fibroblast, but the lamellipodia activated by Rac1 was not observed(Fig. 3B).

2.5 The effect of P92GEF on malignant transformation, migration, invasion and proliferation

We first detected the capacity of transforming NIH3T3 fibroblast by P92GEF in vitro, Dbl-DHPH domain as the positive control, and empty vector as negative control. As shown in Fig.4A, P92GEF could transfect NIH3T3 fibroblast and formed colonies as same as Dbl-DHPH. By contrast, colonies were hardly detected in the negative control, showed that empty vector has the transfecting

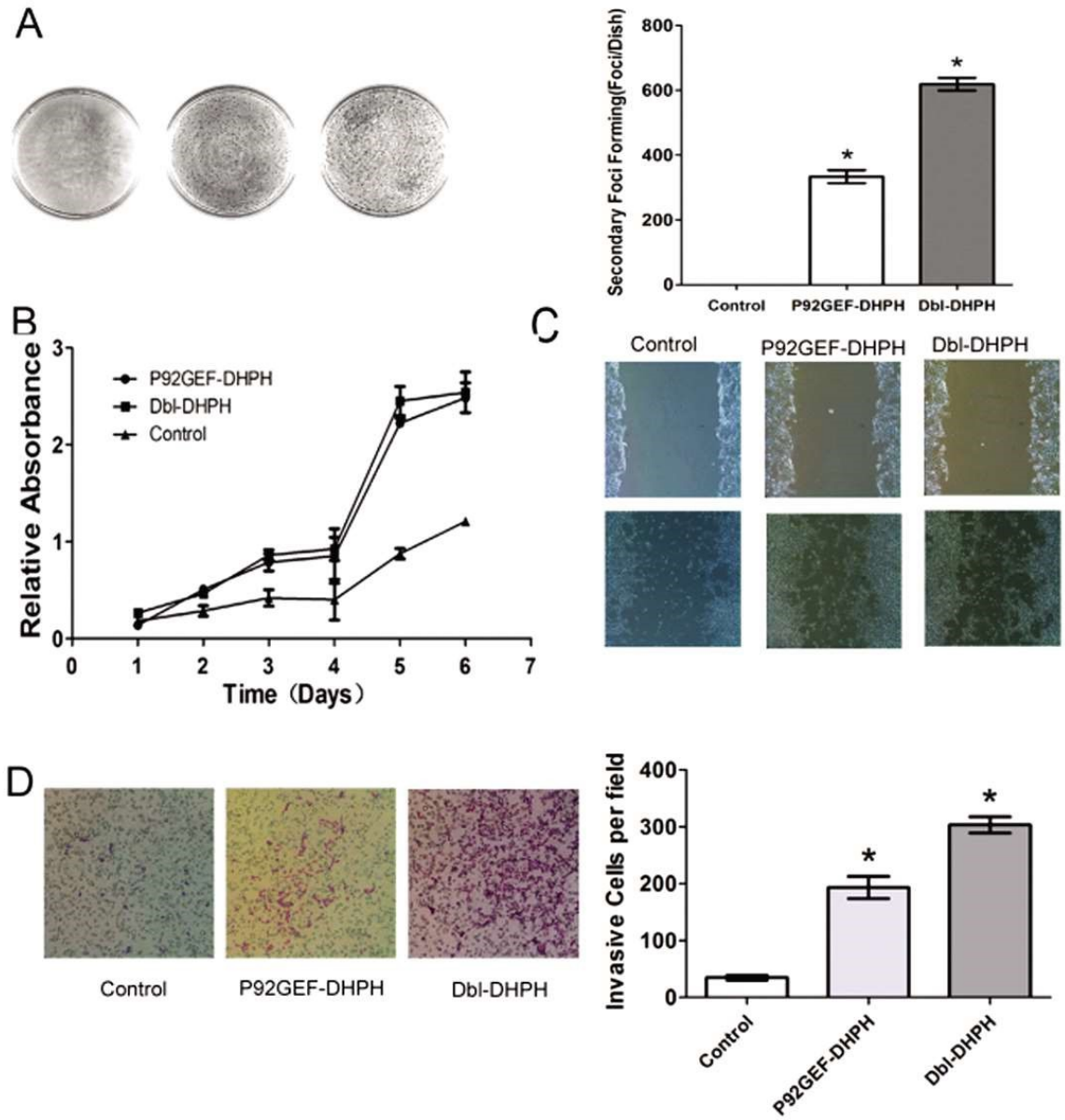


Fig. 4 A. The effect on malignant transformation ability of fibroblast NIH3T3 fibroblast by P92GEF Clone DHPH domain of P92GEF to eukaryotic expression vectors, screen by G418 and transfect the vectors to NIH3T3. Plating and Culturing for focus formation assays.

After 5 days, stain cell and take photos after cell counting.

Figure a means comparison of cloning number pictures; control group is the cell with transfection of empty vector. Figure b means statistical analysis and Graphs after cell counting.

B. Cell proliferation assay: detect cell numbers of NIH3T3 with stable transfection of P92GEF/DHPH, Dbl/DHPH and empty vector by Cell Counting Kit-8 assay in 0, 24, 48, 72, 96, 120h. The data represent means from three separate experiments. *$P < 0.05$.

C. P92GEF promotes NIH3T3 fibroblast migration. Cell migration was measured by the wound-healing assay described in "Materials and methods". The cells were traced for 48 hr after a wound was introduced. Ectopic expression of P92GEF enhanced the migrations of NIH3T3 fibroblast, Dbl as the positive comtrol and empty vector as the negative control. Data are representative of three independent experiments.

D. The effect on invasion ability of NIH3T3 fibroblast by P92GEF.

Plate cells with stable expression of P92GEF/DHPH and Dbl/DHPH in Matrigel-coated transwell. After 24h, take photos after cell counting. Analyze the effect on invasion ability of NIH3T3 fibroblast by P92GEF. Values represent the mean (SEM) 6 SE of three separate experiments. *$P < 0.01$.

ability.

Moreover, the proliferation influence of P92GEF on the NIH3T3 fibroblast was analysised by CCK8 assay kit, and the result was shown in Fig.4B. Compared with Dbl and empty vector, P92GEF displayed the obvious ability of enhancing cell proliferation. In addition, wound-healing assay and transwell assay were carried out separately to observe the migration and invasion ability of P92GEF. In comparison with oncogene Dbl-DHPH as positive control and empty vector as negative control, P92GEF could enhance the invasion ability of NIH3T3 obviously (Fig.4C). As a result of migration test, it was shown that there's unobvious enhancement of P92GEF to cell migration(Fig.4D).

We next tested the cloning forming ability (tumorigenesis in vivo) of P92GEF by soft agar assay. Dbl-DHPH were still used as positive control, empty vector-transfected 293 cells were used as negative control. As the chart shows, compared with empty vector, P92GEF could significantly promote soft agar colony assay of 293cell, as DBL-DHPH(Fig.5).

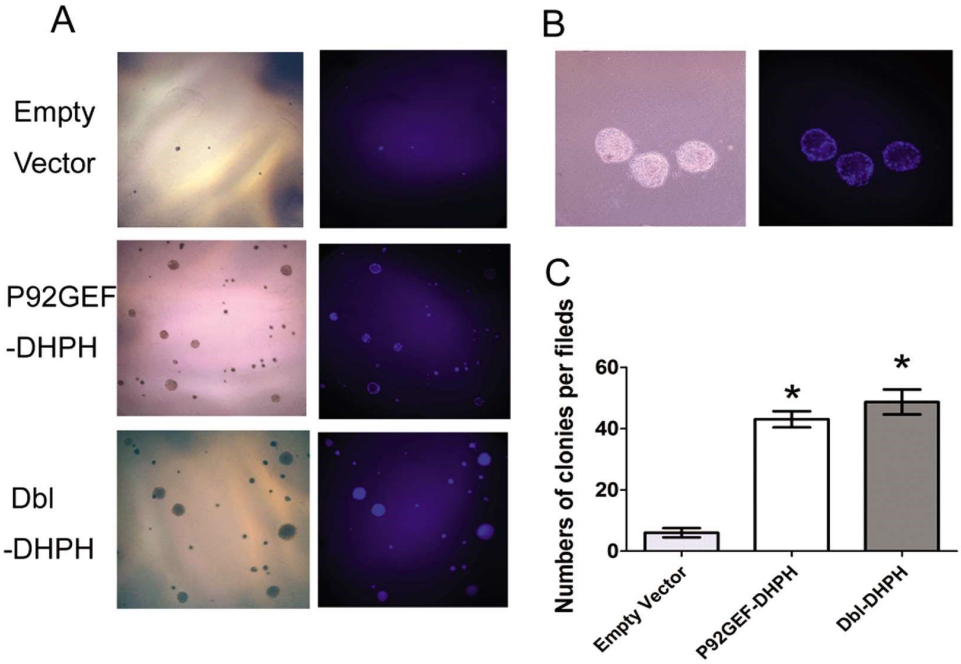


Fig. 5 The soft agar clone formation of 293 cells after 15 days.(A)Soft agar clone were detected with microscope(× 40; Left: white light; right: DAPI); (B)Soft agar clone were detected with microscope(× 40; Left: white light; right: DAPI); (C)Statistical analysis and Graphs after cell clone counting.

2.6 The effect on small molecule transcription factor activity by P92GEF

Transient transfection experiment was carried out to find signal transduction pathways which can be activated by P92GEF for its ability to function as an oncoprotein. The P92GEF vectors and luciferase reporter plasmids containing promoters sensitive to various different signaling pathways were both utilized.

Then the function of P92GEF as oncoprotein in various different pathways was researched. Transient transfection experiments to P92GEF was performed in the HEK293 cell line. Downstream transcription factors chosen as reporter gene were: GAS(γ-interferon activation sequence), ISRE(interferon-stimulated

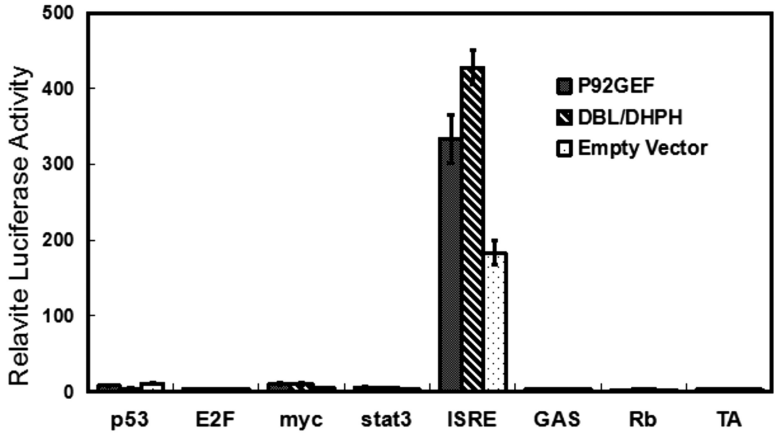


Fig. 6 The effect on transcription factors activity of signaling pathway by P92GEF

The effect on transcription factors activity of signaling pathway by P92GEF was detected by Dual-luciferase reporter assay. In HEK293 with over expression of P92GEF/DHPH, Dbl/DHPH and empty vector, detect the activity of 7 firefly luciferase reporter(Invitrogen), and take a luciferase of negative control plasmid PTA as control, a renilla luciferase vector PRL-TK as internal control. (Describing as in Materials and Methods).

The level of Reporter was expressed by the relative ratio of firefly luciferase activity to renilla luciferase activity. Compared with Dbl/DHPH, there was an obvious increase of myc, ISRE activity by P92GEF, and an increase of MYC P53 activity. Values represent the mean (SEM) 6 SE of three separate experiments. * indicates a significant difference luciferase activity from empty vector ($P < 0.05$).

response element), STAT3 (signal transducer and activator of transcription-3) and Rb, E2F, myc and p53 pathways (Promega). Dual-Lucifrase report gene Assay Kit was used to detect transcrip tion factor activity in the experiment. At first, Transcription factor activity was shown directly by using Firefly luciferase, and errors in experimental result, which was caused by the difference of quantity cells and lysis degree in the experimental group, were re duced by renilla luciferase using as internal control. Each experi mental group was repeated with triplicate samples, us ing Dbl as positive control, and empty vector as negative control. The level of transient were significantly up-regulated p53, myc and ISRE-responsive promoters, by contrast with the positive control (Dbl oncogene). The result revealed that P92GEF probably play a part in MAPK, Jak/Stat pathways by Rho GTPase.

3 Discussion

GEFs regulates cell migration, infiltration and transfer by regulating Rho family members from inactive GDP status to active GTP status and through the recombination of cytoskeleton. Fur thermore, it regulates cell growth cycle and is associated with cell growth and differentiation. In this article, we described P92GEF-a RhoA specific Guanine nucleotide exchange factor that activates Rho GTPase. By analysis of gene sequence and functional domain, we found the gene have a tandem Dbl homology-pleck strin homology (DH-PH) motif functional domain, which be longs to GEFs family. By evolution tree analysis, we found the gene has a low sequence identity with many other Dbl family members but has high sequence identity of DHPH domain with ephexin, which was reported as a typital GEFs mainly expressed in brain[19].

In the research of tissue expression of P92GEF, we did not adopt traditional methodology such as Northern Blot with a digoxigenin labeled probe, instead we applied Realtime PCR to make the detection result achieve partial quantification. This de tection had higher throughput and was more accurate than Northern Blot. The results showed lungs-specific expression of P92GEF was the highest.

Furthermore, we found that P92GEF has GEF activity to wards RhoA and Rac1 in small G proteins in the research of GEF activity detection in vivo. However, there was not obvious forma-tion of lamellipodia after the increase of stress fiber by the cy-toskeleton immunofluorescence test. So we deduced that the GEF activity of P92GEF was mainly on RhoA, and the regulation of cy-toskeleton mainly reflected the generation of stress fiber [21]. In the research of GEF-ephexin which has a higher sequence identity with P92GEF, it indicated that RhoA, Cdc42 and Rac1 all had activity in the GEF activity detection in vivo. However, Stress fiber induced by RhoA was more significant than lamellipodia or fillapodia in the cytoskeleton immunofluorescence test, which was similar to our research.

Through NIH3T3 cloning experiment, we found P92GEF has ability of malignant transformation, which was characteristic of typical oncogene. Then we detected the cell migration, invasion, multiplication affected and soft agar colon fomation by P92GEF, and found that it had stimulative effect on cell multiplication and invasion to a certain extent. But the effect on cell migration was not obvious.

The analysis of regulation of downstream transcription factor activity is to find out the relevant signal pathway of P92GEF. Then we found there was an obvious increase of transcription factor activity in myc, GAS, STAT3 and ISRE. Myc is a well known oncogene. In the past research it was found that translocation between C-myc loci and Ig loci would make C-myc shift to highly active transcription area. Then a rearrangement gene with highly active transcription was set, C-myc transcription started, C-myc expression increased, malignant cells were promoted, the result was malignancy. At present, it was considered that there was am plification or over expression of myc in Gastric cancer, breast cancer, colon cancer, cervical cancer, Hodgkin's disease and head tumor. The experimental result prompted that P92GEF maybe have some kind of relationship with myc gene translocation, change cell cycle by increasing the transcription factor activity of myc, and cause the malignant proliferation of cancer cells. (re vealing for the first time that in human fibroblasts Rac1 exerts control on proliferation through c-myc phosphorylation. Thus Rac1 activates proliferation of normal fibroblasts through stimula tion of c-myc phosphorylation without affecting ERK1/2 activity[22, 23].

Moreover, the up regulation of GAS, ISRE and STAT3 caused by P92GEF prompted that P92GEF maybe have some kind of relationship with JAK-STAT pathway and induction of transcriptional regulation of MHCI [24, 25]. Cross talk between Rho and stat3 showed that the mutational activation of Rac1 and Cdc42 results in Stat3 activation, which occurs in part through the up-regulation of IL6 family cytokines that in turn stimulates Stat3 through the Jak kinases [26], and STAT3 represents an essential ef fector pathway of Rho GTPases in regulating multiple cellular functions including actin cytoskeleton reorganization, cell migra tion, gene activation, and proliferation [27]. Also, RhoA also can efficiently modulate Stat3 transcriptional activity by inducing its simultaneous tyrosine and serine phosphorylation. So, P92GEF is the upstream regulator of Rho-JAK/STAT pathway[28].

P 92GEF would participate in several biological process such as Cell proliferation, differentiation, apoptosis and immune regula tion, etc., but the concrete pathway still needed to be stud ied. In brief, the research of transcription factor activity provided us a reliable theoretical foundation to look for drug targets treating cancers.

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鸟苷酸交换因子 P92GEF 通过靶向调控 RhoA 抑制肿瘤细胞增殖侵袭 *

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摘要：Dbl 家族鸟苷交换因子（GEFs）是 Rho 家族蛋白发生恶性转化的主要调控单位，它通过使 Rho 蛋白从无活性的 GDP 形式转换为 GTP 形式的 Rho 蛋白而发挥作用，参与细胞骨架重排，细胞的生长和活力。P92GEF 是一 GEFs 家族分子。本研究通过 Real time PCR 对 P92GEF 在人体 48 种正常组织中的表达情况进行了测定；GST-pulldown 技术对 P92GEF 的体内 GEF 活性进行了检测；双荧光素酶报告基因检测技术对下游小分子进行转录因子活性检测；应用免疫荧光双染标记法完成了高表达 P92GEF 对正常细胞骨架形态的影响；在细胞表型实验中分别使用 CCK8 法、Transwell 法及软琼脂克隆形成实验检测了高表达 P92GEF 对细胞增殖侵袭迁移及体外成瘤能力的影响。研究结果显示 P92GEF 有 841 个氨基酸，具有典型的 Dbl 家族分子结构域，在肺组织中表达量最高，能够促使正常成纤维细胞中的应力纤维（stress fiber）增多，P92GEF 转染的 NIH3T3 细胞可以独立生长和形成继发性病灶，同时促使细胞增殖，侵袭及克隆形成能力增强，体外转录因子活性检测发现该基因可能与 JAK/STAT 通路有关。因此，P92GEF 是一个典型的鸟苷交换因子家族分子，能激活 Rho 家族分子 RhoA，具有明显的癌基因特征。

关键词：鸟苷酸交换因子；P92GEF；Dbl 家族；Rho 家族；癌基因

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