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Cell adaptation to activated FGFR3 includes Sprouty4 up regulation to inhibit the receptor-mediated ERKs activation from the endoplasmic reticulum

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ABSTRACT

The kinase activity of the thanatophoric dysplasia type II–fibroblast growth factor receptor 3 mutant (TDII–FGFR3) hampers its maturation. As a consequence, the immature receptor activates extracellular regulated kinases (ERKs) from the endoplasmic reticulum (ER), which leads to apoptosis. On the other hand, in stable TDII–FGFR3 cells receptor biosynthesis is restored and ERKs are activated from the cell surface. To identify potential mediators of cell adaptation to the activated receptor we investigated gene products that are differently regulated in TDII and wild-type FGFR3 cells. cDNA representational difference analysis reveals Sprouty4 up regulation in the TDII–FGFR3 cells. Interestingly, Sprouty4 inhibits the TDII–FGFR3-mediated ERKs activation from the ER, but fails to suppress ERKs activation from cell surface. We conclude that cell adaptation to activated FGFR3 include Sprouty4 activity, which silences the premature receptor signaling and suppress apoptosis.

1. Introduction

Fibroblast growth factor receptor 3 (FGFR3) is a member of the tyrosine kinase FGFR family [1] and plays an important role in a variety of processes of embryonic development and tissue homeostasis [2,3]. Mutations in the kinase activation loop domain of the FGFR3 are associated to bone disorders and cancer [4], diseases with opposite patho-physiological profiles. We have previously shown that the kinase activity associated to the K650E substitution of the thanatophoric dysplasia type II (TDII)–FGFR3 affects receptor trafficking and results both in the accumulation of the immature 120 kDa TDII glycomer in the endoplasmic reticulum (ER) and apoptosis [5,6]. Accordingly, apoptosis has been detected in chondrocytes from TD fetuses in vivo and in the ATDC5 chondrogenic line in vitro [7,8]. On the contrary, enlarged brains with increased proliferation and decreased apoptosis of the cortical progenitors it was shown in mice carrying the FGFR3 with the K644E mutation [9] indicating how the cellular environment may affect FGFR3 behavior. Furthermore, we have recently shown that, in transient expression assays, the immature TDII receptor glycomers transduce the signal from the ER thus leading to the activation of the extracellular regulated kinase (ERK) proteins through FRS2α and PLC-gamma independent pathways [10]. Differently, in stable cell lines where both immature and mature FGFR3 phosphorylated glycomers are detected, ERKs are activated from the cell surface through the canonical FRS2α/Grb2/Ras/MAPK pathway [3,10].

The first Sprouty gene was identified in mutational screening for genes that modulate FGF signaling during tracheal and eye development in *Drosophila* [11]. Furthermore, Sprouty was found to be a ligand-inducible antagonist of receptor tyrosine kinase (RTK) signaling in *Drosophila* by its ability to suppress Ras/MAPK pathway [12]. Four mammalian genes (Spry 1-4) encoding proteins that share a highly conserved cystein-rich domain at the carboxyl terminus that allow their inhibitory role on MAPK pathway have been identified [13]. Expression of Sprouty4 is induced by FGF signaling and it has been proposed that Sprouty4 protein functions as FGF-induced feedback inhibitor [14]. Furthermore, Sprouty2 and Sprouty4 are essential for embryonic morphogenesis and regulation of FGF signaling [15]. It has been reported that Sprouty2 disturbs FGFR3 degradation in the TDII condition

Abbreviations: FGFR3, fibroblast growth factor receptor 3; TDII, thanatophoric dysplasia type II; cDNA-RDA, cDNA representational difference analysis; ERK, extracellular regulated kinases; ER, endoplasmic reticulum

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suggesting that Sprouty2 plays a role in the pathogenesis of the FGFR3-mediated skeletal dysplasias [16]. In addition, it was shown that Sprouty4 suppresses vascular epithelial growth factor (VEGF)-induced Ras-independent activation of Raf1 by direct binding to Raf1 through its carboxyl-terminal cystein-rich domain [17].

In this study, we have investigated on genes that were differently regulated in TDII versus wild-type FGFR3 stable cell clones. We find that Sprouty4 is up regulated exclusively in the TDII–FGFR3 cells and it inhibits the TDII-induced ERKs activation from the ER. We suggest a role by Sprouty4 in the adaptation process to the activated FGFR3.

2. Materials and methods

2.1. Cells and transfection

Hek239 cells-derived FGFR3 cell clones were generated as previously described [5]. Transfections were performed with Lipofectamine-2000 (Invitrogen) as described [5,6]. FGFR3 constructs were previously described [5,6].

2.2. cDNA representational difference analysis (cDNA-RDA)

cDNA-RDA was originally described by Hubank and Shatz [18]. Briefly: polyA$^+$ RNA was obtained from HEK293 cells, two independent HEK293-derived TDII–FGFR3 clones (TDII cl. 77 and 55), two independent wild-type FGFR3 clones (wt clones 3 and 15) and one G418-resistant HEK293 cell clone. The first-strand cDNA was synthesized by SuperScript (Invitrogen). The second-strand cDNA was synthesized in RT2 buffer, βNAD, *Escherichia coli* DNA ligase, *E. coli* DNA polymerase, dNTP's and incubated for 2 h at 15 °C and then for 1 h at 22 °C. Double stranded (ds) cDNA was digested with Dpn II at 37 °C for 1.5 h. The R-24mer and R-12mer linkers (R-24mer 5′-AGCACTCTCCAGCCTCTCACCGCA-3′; R-12mer 5′-GATCTGCGGTGA-3′) were legated/annealed to the Dpn II restricted ds cDNA's. PCR was performed in PCR machine (Biometra). To generate "representations", 200 μl PCR reactions were set up for each representation. 40 μg of each representation were digested with Dpn II to originate the cut "drivers" representations. "Tester" was prepared by purifying 20 μg digested representation on a 1.2% TBE gel. J-24mer and J-12mer oligos (J-24mer 5′-ACCGACGTCGACTATCCATGAACA-3′; J-12mer 5′-GATCTGTTCATG-3′) were ligated to 2 μg of purified "tester" DNA. PCR was performed overnight. Subtractive hybridization was performed by mixing 40 μg of "driver" to 0.4 μg of J-ligated "tester" and extracted with phenol chloroform. For each subtraction, 4 PCR reactions were performed using 20 μl of subtractive hybridization. The first difference product was obtained by digesting 20 μl of the PCR products with Mung Bean nuclease at 30 °C for 35 min followed by 4 PCR reactions using 20 μl of DNA as template with J-24mer primer. The first difference product was digested with Dpn II to remove the J oligos. For the second and third subtractive hybridization, 50 ng of N-oligos were used (N-24mer 5′-AGGCAACTGTGCTATCCGAGGGAA-3′; N-12mer 5′- GATCTTCCCTCG-3′). The subtractive and amplification steps were performed as above.

2.3. Sprouty4 cDNA cloning

Sprouty4 was cloned by PCR from HEK293–FGFR3–TDII cl. 77 cells using the following DNA primers: 5′-ACAAAGCTTATGCTTGTGACCCTGCAGC-3′ and 5′-ACATCTAGATAAAACCTCTGACCTTGC-3′. The 1078 bp PCR product was cloned into PCRII topo vector (Invitrogen) and transfected into DH5α competent cells. A Myc tag was added in frame to the 3′ end of Sprouty4 cDNA.

2.4. Real time PCR

The level of Sprouty4 mRNA was measured by real-time quantitative RT-PCR (Life Technologies) using the PE ABI PRISM 7700 Sequence Detection System (Applied Biosystems). Expression of the housekeeping gene GAPDH was measured in parallel as an endogenous control. The sequences of forward and reverse primers and of the TaqMan fluorogenic probes, as designed by the Primer Express 1.5 software, were Sprouty4 (GenBank No. NM030964): forward 5′-ACCTGCCCCGGCTTCA-3′, reverse 5′-AGGTCCTGGACTGTACGGAGAA-3′, probe 5′-FAM-CAGCTCCTCAAAGGCCCCTAGAAGCC-TAMRA-3′; GAPDH (GenBank No. NM008084): forward 5′-TGTGTCCGTCGTGGATCTG-3′, reverse 5′-GATGCCTGCTTCACCACCTT-3′, probe 5′-TET-TGATGTCATCATACTTGGCAGGTTTCTCCA-TAMRA-3′.

The probes were located at the junction between two exons. Relative transcript levels were determined from the relative standard curve constructed from stock cDNA dilutions and divided by the target quantity of the calibrator according to the manufacturer's instructions.

2.5. Immunoblot and antibodies

Transfected cells were lysed in RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) in the presence of a mixture of protease and phosphatase inhibitors, clarified by centrifugation and subjected to immunoblot; FGFR3 proteins were resolved on 7% tricine gels as previously described [10]. The following antibodies were used: anti-HA and anti-Myc (Roche); anti-Phospho tyrosine 4G10 (UpState). Sprouty4 antibody was kindly provided by Dr. Akihiko Yoshimura (Kyushu University). Goat anti-Sprouty4 antibody (L-17) was purchased from Santa Cruz. Antibodies detecting ERKs (C-14) from Santa Cruz; phospho-ERKs (Biosource); anti-GFP (Abcam), anti-tubulin (Sigma). Immunoprecipitation (IP), and whole cell extracts (WCE) were performed as previously described [5].

3. Results and discussion

The incomplete maturation of the FGFR3 bearing the highly activating K650E mutation (TDII mutation) is associated to the premature signaling from the ER by the immature 120-kDa receptor glycomers. Furthermore, the premature signaling from the ER is associated to apoptosis [5]. Accordingly, efforts to obtain stable cell lines expressing the TDII–FGFR3 gave only a limited number of positive cell clones. On the contrary, cell lines expressing the wild-type FGFR3 were easily achieved suggesting that the selection process ending in cell adaptation to the activated TDII–FGFR3 required further steps.

To identify potential mediators of the cell adaptation to the activated TDII–FGFR3, we have performed the PCR-coupled subtractive process of representational difference analysis (RDA) on the HEK293-derived TDII and wild-type FGFR3 cell clones. Furthermore, to rule out that any difference in gene regulation could be the result of the G418-selection process, the cDNA-RDA was performed on HEK293 cells that express the G418 resistance only. After three rounds of subtractive hybridization/amplification, we have obtained the partial DNA sequences of gene products that were differently regulated in the analyzed cell lines. Search through BLAST database allowed us to identify a number of genes that were differentially expressed in the TDII versus the wild-type FGFR3 cells (the identified genes are listed in Table 1). Among these products, Sprouty4 resulted up regulated in the TDII cells. We have focused our attention on Sprouty4 because of its role as a feedback-induced antagonist of FGF signaling in *zebrafish* during

Table 1
Genes that are differentially expressed in stable TDII–FGFR3 versus wild-type FGFR3 cells.

Accession number	Up regulated genes
NM030964	Sprouty4
NM004078	Cystein and glycine rich protein; CRP-1
NM001986	Ets variant gene 4; ETV-4
	Down regulated genes
BC036472	Protein kinase beta-1 PKC beta 1
NM013906	A disentegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif; ADAMTS-1
U52219	Melatonin related receptor; GPR50
NM021988	Zinc finger protein 6; ZNF6
M86181	Human prosaposin; hPSAP
BC0003322	Ubiquitin carboxyl-terminal esterase UCHL1
U19720	Reduced folate carrier L1; RFC/SLC19A1

embryonic morphogenesis [14,19]. Therefore, according to our hypothesis that the signaling from the ER by the TDII variant should be switched off to allow the TDII–FGFR3 cells selection, Sprouty4 represented a reasonable candidate acting as inhibitor of the TDII–FGFR3 intracellular signaling. The expression level for the other members of the Sprouty family has not been examined. The reason to ascribe Sprouty4 as a specific intracellular silencer of the TDII signaling is because Sprouty4 suppresses the Ras-independent ERKs activation but not the Ras-dependent ERKs activation [17]. To this regard, we have previously reported that the TDII receptor activates ERKs from the ER through an FRS2α and PLCγ-independent pathway [10] therefore, the transduction pathway from the ER is different from the one triggered by the mature FGFR3 from the cell surface that include FRS2α/Grb2/Sos/Ras/Raf/MAPK activation [3].

A first series of experiments were aimed to confirm the Sprouty4 up regulation in the TDII–FGFR3 versus the wild-type cells. Initially, real time PCR was performed on TDII versus wild-type cells. As shown in Fig. 1, a two- to threefold Sprouty4 increase was observed in the TDII cells compared to the wild-type and the parental G418-resistant HEK293 cells. The level of Sprouty4 in the TDII clone 77 is slightly higher probably due to clonal variation. Furthermore, to determine the Sprouty4 protein level in the TDII cells, immunoprecipitation experiments were performed with Sprouty4 specific antibody. A faint band of approximately 35 kDa, indicating the relatively low Sprouty4 endogenous level, was detected in the TDII clone 77 (Fig. 2) as well as in two other independent TDII

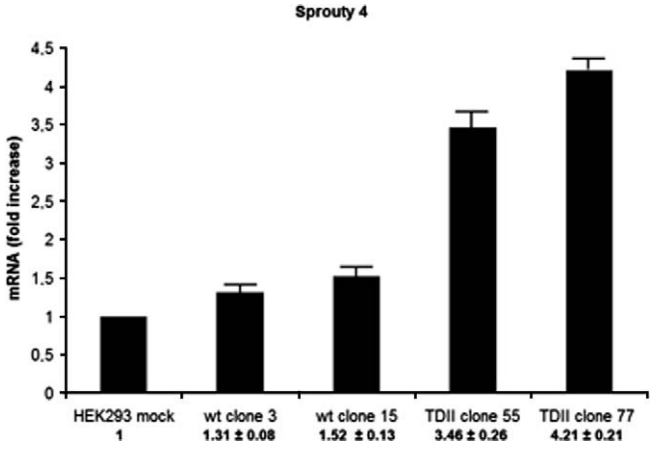

Fig. 1. Sprouty4 RNA expression levels in TDII versus wild-type FGFR3 cells. Real time PCR shows Sprouty4 mRNA expression fold increase in untransfected-G418 resistant (mock), wt-FGFR3 clones 3 and 15, and TDII–FGFR3 clones 55 and 77 cells.

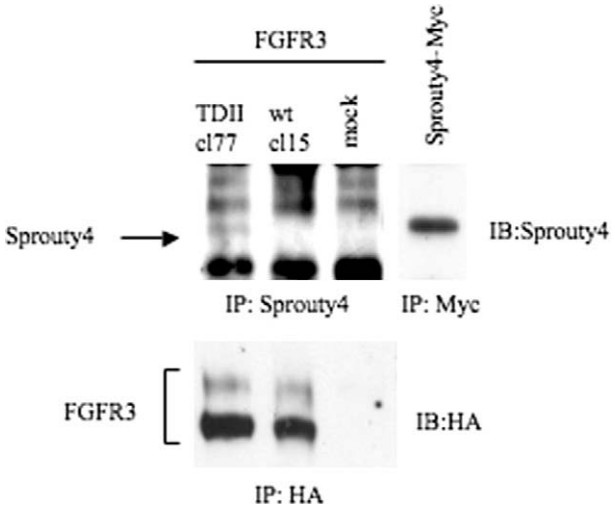

Fig. 2. Sprouty4 protein level. TDII clone 77, wild-type clone 15 and mock HEK293 cells were immunoprecipitated with anti-Sprouty4 antibody (a generous gift by Dr. Akihiko Yoshimura). As a positive control Sprouty4-Myc was independently transfected into HEK293 cells and the Myc antibody used for immunoprecipitation. Immunodetection was performed with Sprouty4 antibody. The same protein extracts were immunoprecipitated and hybridized with anti-HA antibody to show the 120 and 130 kDa FGFR3 forms in the cell clones.

clones analyzed (not shown). Sprouty4 protein remained undetected in the wild-type FGFR3 as well as in the parental G418-resistent HEK293 cells.

Next we examined whether in the stable TDII–FGFR3 cells the ERKs were activated by the mature, fully glycosylated FGFR3 on cell surface, or by the ER-located phosphorylated glycomers. For the purpose, cells were treated with monensin, which is a drug known to block the secretory pathway thus abolishing the presence of the receptors on the cell surface [6]. In Fig. 3A, we show that monensin completely abolishes ERKs activation in the TDII clone 77 (lane 5). To determine if the activated immature glycomers, derived from FGFR3 with a different mutation, were signaling as well from intracellular compartments, monensin treatment were performed in the SADDAN-FGFR3 clone 3 (SADD cl. 3). The SADDAN cells express a different, highly activated FGFR3 mutant, who is associated to the severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN, K650M substitution). The result shown in Fig. 3A (lane 7) confirms that the SADDAN-FGFR3 behaves similarly to the TDII variant [10]. Furthermore, as a control, we explored if monensin could affect both FGFR3 biosynthesis and phosphorylation. For the purpose, the wild-type cl. 15, TDII cl. 77 and SADDAN cl. 3 were treated with monesin. Fig. 3B shows that neither FGFR3 biosynthesis nor phosphorylation are affected by monensin treatment. Altogether these results indicate that in the stable TDII/SADDAN cells the ERKs are activated from the cell surface by the highly activated FGFR3 even if the immature 120 kDa phosphorylated glycomers are still present as shown in Fig. 3B.

To investigate on the Sprouty4 functional role as a modulator of the FGFR3 signaling, the Sprouty4 cDNA amplified by PCR from the TDII clone 77 cDNA library, was obtained. Subsequently, the Myc tag was inserted in frame to the 3′ end of Sprouty4 and the expression vector named Sprouty4-Myc was generated to perform a series of transfection experiments.

To determine if Sprouty4 could suppress ERKs activation by the FGFR3 mutants from cell surface, Sprouty4-Myc was transfected into the TDII and SADDAN cell clones. As predicted, Sprouty4 over-expression did not affect ERKs activation in both cell lines (Fig. 4A) indicating that indeed, Sprouty4 cannot suppress the

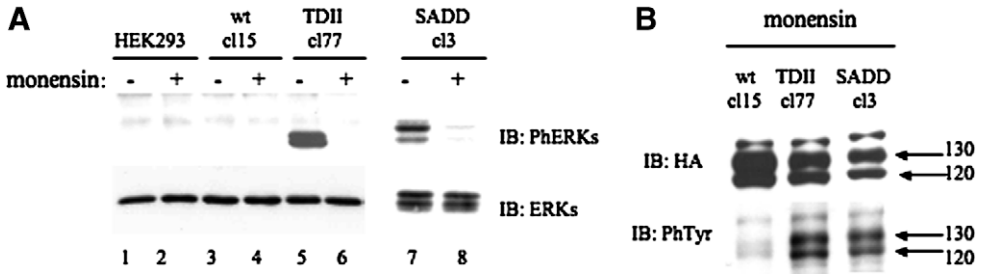

Fig. 3. ERKs are activated from the cell surface by the TDII/SADDAN-FGFR3 in stable clones. (A) The parental HEK293, wild-type cl. 15, TDII cl. 77 and SADD cl. 3 cells were analyzed for ERKs activation in the presence (+) or absence (−) of 3 μM monensin for 5 h. Twenty micrograms of whole cell extracts were loaded in each lane and immunoblot with anti-Ph-ERK antibody was performed. Filters were reprobed with the indicated antibody. (B) Neither FGFR3 biosynthesis nor phosphorylation are affected by monensin treatment. The same cells as in (A) were treated with monensin for 5 hours. Cells exhibit both immature (120 kDa) and mature (130 kDa) glycomers as indicated. Only the TDII and SADDAN cells exhibit both mature and immature phosphorylated glycomers.

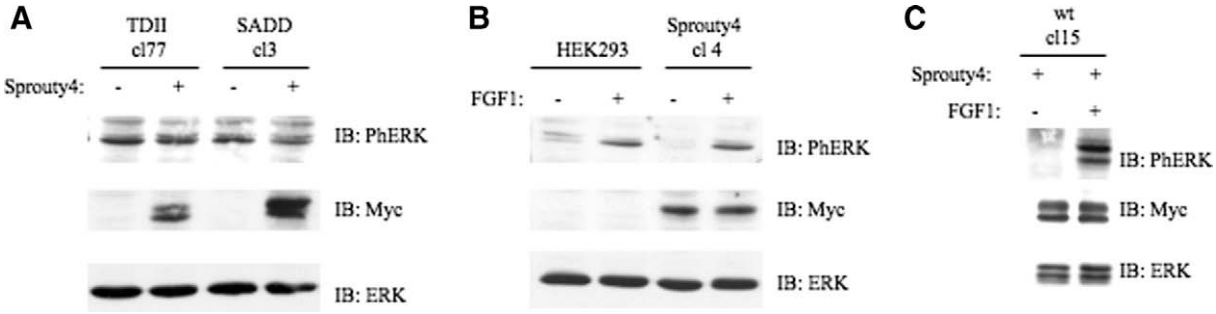

Fig. 4. Sprouty4 does not suppress ERKs activation from cell surface. (A) TDII and SADDAN cell clones were transfected with Sprouty4 as indicated. ERKs activation remains unchanged indicating that Sprouty4 does not affect the FGR3-mediated signal transduction from cell surface. (B) Parental HEK293 and the HEK293-derived Sprouty4 cells were stimulated for 15 min with FGF1. Activated ERKs are detected upon FGF treatment in both cells. The data confirms that the endogenous FGFR pathway is unaffected by Sprouty4. (C) In the presence of Sprouty4, the wt cl. 15 cells still exhibit ERKs activation upon FGF1 treatment.

FGFR3 signaling from the cell surface. A further confirmation that Sprouty4 did not affect both TDII and SADDAN signaling from the cell surface was obtained by analyzing FRS2α. Results showed that FRS2α remained in its activated conformation in the presence of Sprouty4 (not shown). Further experiments were performed to investigate whether Sprouty4 could affect signaling by other endogenous FGFR exposed on HEK293 cell surface. To this regard it is worth mentioning that we did not detect endogenous FGFR3 in HEK293 cells [5]. Only low levels of endogenous FGFR2 were detected in the HEK293 cells (unpublished observation) however, we cannot exclude a low expression level of both FGFR1 and FGFR4. For the purpose, HEK293 and the HEK293-derived Sprouty4 cells (Sprouty4 cl. 4) were stimulated with FGF1 and the ERKs were analyzed. In Fig. 4B it is shown that ERKs are activated upon FGF1 stimulation even in the presence of Sprouty4. These data confirm that the FGFR-mediated signaling from cell surface is unaffected by Sprouty4. Furthermore, the FGFR3 protein remains undetected in the Sprouty4 cell line indicating that Sprouty4 does not up-regulate FGFR3 expression. In addition, we show that Sprouty4 does not inhibit ERKs activation by FGF1 in the wt-FGFR3 cells thus confirming that Sprouty4 cannot abrogate the FGFR3-mediated signaling from the cell surface (Fig. 4C).

Considering that Sprouty4 suppresses ERKs activation in the Ras-independent pathway [17], we have investigated on whether Sprouty4 could inhibit the ERKs activation triggered by the immature TDII and SADDAN receptors, in transient transfection assays. For this purpose, Sprouty4 and the TDII or the SADDAN variants were co-transfected and proteins were analyzed 48 h later. Fig. 5A shows that Sprouty4 inhibits ERKs activation triggered by both the TDII and the SADDAN receptors (lanes 3 and 4). Furthermore, the phosphorylation level of the TDII receptor (and SADDAN receptor, not shown) remains unchanged in the presence of Sprouty4 (Fig. 5B) indicating that Sprouty4 operates downstream the Tyrosine phosphorylation event.

All together our data suggest that a physiological role played by Sprouty4 could be to inhibit the signaling from the ER during the cell adaptation process to activated TDII/SADDAN-FGFR3. We have previously shown that the premature signaling from the ER by the activated FGFR3 triggers an apoptotic response, which could be consequent to a stress of the ER. We propose that suppression of apoptosis is required to allow the positive selection of TDII/SADDAN cell clones, a process that results from both adaptations to activated FGFR3 and resistance to G418. The potential relevance of cell adaptation to activated FGFR3 could be related to the pathogenesis of the FGFR3-related human cancers, as suggested by our previous observation that the KMS11 and OPM-2 multiple myeloma cells, although presenting a strong FGFR3 auto phosphorylation, fail to activate proteins (STATs) that are indeed activated by the immature TDII–FGFR3, in transient expression, from intracellular compartments [5].

It remains to determine whether inhibition of Sprouty4 could re establish the apoptotic pathway in stable TDII cells. A partial answer derives from the observation that transfection of TDII–FGFR3 in stable TDII cell clone does not allow ERKs activation in the presence of monensin and apoptosis is undetected (not shown) thus suggesting the irreversibility of the adaptation process. However, more experiments such as Sprouty4 knockdown are required to determine the precise role by Sprouty4 on cell adaptation to activated FGFR3.

In conclusion, Sprouty4 up-regulation may be initially triggered by the activated FGFR3. Subsequently, Sprouty4 acting as a feedback inhibitor suppresses the signaling from the ER. Such a model

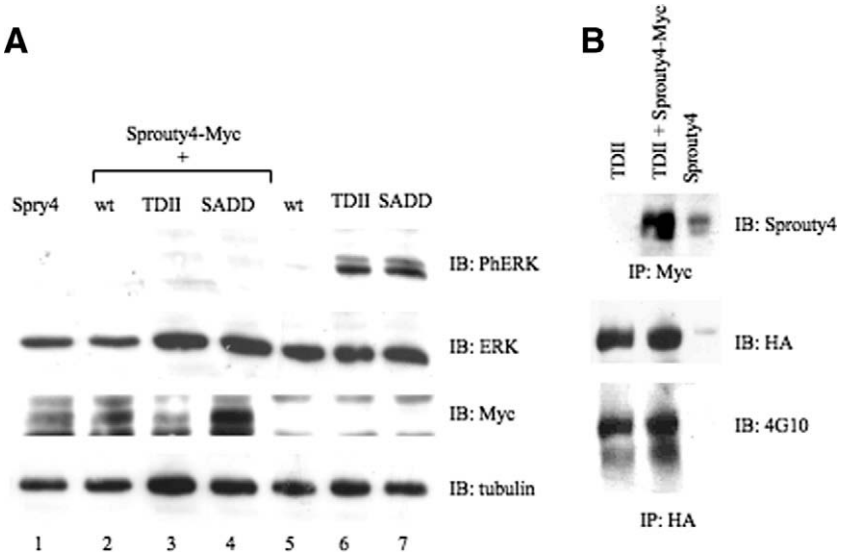

Fig. 5. ERKs activation from the ER by TDII–FGFR3 is abolished in the presence of Sprouty4. (A) Co-transfection of TDII–FGFR3 with Sprouty4-Myc followed by immunoblot (IB) with the indicated antibody shows that ERKs activation by TDII and SADDAN-FGFR3 is drastically reduced in the presence of Sprouty4 (lanes 3 and 4). Sprouty4 alone does not induce ERKs activation (lane 1). (B) Sprouty4 does not co-precipitate (top) with FGFR3–TDII and is not phosphorylated (bottom) by the FGFR3–TDII.

could be associated to an increased cellular proliferation by the TDII/SADDAN-FGFR3, in line with the report by Thomson et al. [9] in which it is shown the increased Sprouty levels associated to increased proliferation and decreased apoptosis of cortical progenitors of K644E mice.

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References

[1] Dailey, L., Ambrosetti, D., Mansukhani, A. and Basilico, C. (2005) Mechanisms underlying differential responses to FGF signaling. Cytokine Growth Fact. Rev. 16, 233–247.
[2] Szebenyi, G. and Fallon, J.F. (1999) Fibroblast growth factors as multifunctional signaling factors. Int. Rev. Cytol. 185, 45–106.
[3] Schlessinger, J. (2000) Cell signaling by receptor tyrosine kinases. Cell 103, 211–225.
[4] Ornitz, D.M. and Marie, P.J. (2002) FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. Gene Dev. 16, 1446–1465.
[5] Lievens, P.M.-J. and Liboi, E. (2003) The thanatophoric dysplasia type II mutation hampers complete maturation of fibroblast growth factor receptor 3 (FGFR3) which activates signal transducer and activator of transcription 1 (STAT1) from the endoplasmic reticulum. J. Biol. Chem. 278, 17344–17349.
[6] Lievens, P.M.-J., Mutinelli, C., Baynes, D. and Liboi, E. (2004) The kinase activity of fibroblast growth factor receptor 3 with activation loop mutations affects receptor trafficking and signaling. J. Biol. Chem. 279, 43254–43260.
[7] Legeai-Mallet, L., Benoist-Lasselin, C., Delezoide, A., Munnich, A. and Bonaventure, J. (1998) Fibroblast growth factor receptor 3 mutations promote apoptosis but do not alter chondrocyte proliferation in thanatophoric dysplasia. J. Biol. Chem. 273, 13007–13010.
[8] Harada, D., Yamanaka, Y., Ueda, K., Nishimura, R., Morishima, T., Seino, Y. and Tanaka, H. (2007) Sustained phosphorylation of mutated FGFR3 is a crucial feature of genetic dwarfism and induces apoptosis in the ATDC5 chondrogenic cell line via PLCγ-activated STAT1. Bone 41, 273–281.
[9] Thomson, R.A., Pellicano, F. and Iwata, T. (2007) Fibroblast growth factor receptor 3 kinase domain mutation increases cortical progenitor proliferation via mitogen-activated protein kinase activation. J. Neurochem. 100, 1565–1578.
[10] Lievens, P.M.-J., Roncador, A. and Liboi, E. (2006) K644E/M FGFR3 mutants activate Erk1/2 from the endoplasmic reticulum through FRS2α and PLCγ-independent pathways. J. Mol. Biol. 357, 783–792.
[11] Kim, H.J. and Bar-Sagi, D. (2004) Modulation of signaling by Sprouty: a developing story. Nat. Rev. Mol. Cell Biol. 5, 441–450.
[12] Reich, A., Sapir, A. and Shilo, B. (1999) Sprouty is a general inhibitor of receptor tyrosine kinase signaling. Cell 96, 655–665.
[13] Mason, J.M., Morrison, D.J., Basson, M.A. and Licht, J.D. (2006) Sprouty proteins: multifaceted negative-feedback regulators of receptor tyrosine kinase signaling. Trends Cell Biol. 16 (1), 45–54.
[14] Fürthauer, M., Reifers, F., Brand, M., Thisse, B. and Thisse, C. (2001) Sprouty4 acts in vivo as a feedback-induced antagonist of FGF signaling in zebrafish. Development 128, 2175–2186.
[15] Minowada, G., Jarvis, L.A., Chi, C.L., Neubuser, A., Sun, X., Hacohen, N., Krasnow, M.A. and Martin, G.R. (1999) Vertebrate sprouty genes are induced by FGF signaling and can cause chondrodysplasia when overexpressed. Development 126, 4465–4475.
[16] Guo, C., Degnin, C.R., Laederich, M.B., Lunstrum, G.P., Holden, P., Bihlmaier, J., Krakow, D., Cho, Y.-J. and Horton (2008) Sprouty2 disturbs FGFR3 degradation in thanatophoric dysplasia type II: a severe form of human achondroplasia. Cell Signal. 20, 1471–1477.
[17] Sasaki, A., Taketomi, T., Kato, R., Saeki, K., Nonami, A., Sasaki, M., Kuriyama, M., Saito, N., Shibuya, M. and Yoshimura, A. (2003) Mammalian Sprouty4 suppresses Ras-independent ERK activation by binding to Raf1. Nat. Cell Biol. 5, 427–432.
[18] Hubank, M. and Shatz, D. (1994) Identifying differences in mRNA expression by representational difference analysis of cDNA. Nucl. Acid Res. 22, 5640–5648.
[19] Taniguchi, K., Ayada, T., Ichiyama, K., Kohno, R., Yonemitsu, Y., Minami, Y., Kikuchi, A., Maehara, Y. and Yoshimura, A. (2007) Sprouty2 and Sprouty4 are essential for embryonic morphogenesis and regulation of FGF signaling. B.B.R.C. 352, 896–902.