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Assignment of human *sprouty 4* gene to chromosome segment 5q32~33 and analysis of its pattern of expression

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Abstract

The human *sprouty 4* (*SPYR4*) gene was localized to chromosome band 5q32~33 by screening the Stanford radiation hybrid G3 panel using a *SPRY4*-specific primer pair for PCR. Northern blot analysis revealed two different mRNAs (5 kb and 2 kb) in liver, skeletal muscle, heart, lung, kidney, spleen, placenta and small intestine. Reverse transcriptase-PCR analysis showed that *SPYR4* was expressed in all tested tissues to different levels.

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Introduction

The signals that regulate cell fate are precisely controlled during development to avoid errors, while feedback interaction can impart precision, robustness and versatility to intercellular signals (Freeman 2000). In both developing *Drosophila* trachea and murine lung, a fibroblast growth factor (FGF) signalling pathway is reiteratively used to pattern successive rounds of branching, and the pathway is modified at each stage of branching by feedback regulations (Metzger and Krasnow 1999). *Drosophila sprouty* (dSPRY) was first identified as an inducible gene for an FGF antagonist which encodes a cysteine-rich protein that nonautonomously blocks the FGF pathway in a competitive fashion. Null mutation in dSPRY results in increased tracheal branching (Hacohen *et al*. 1998).

Recent research has shown that Sprouty is involved in the epidermal growth factor (EGF) pathway too; it might be a general receptor tyrosine kinase inhibitor (Reich *et al*. 1999). Correspondingly, Sprouty was found to be involved in multiple biological processes, such as brain patterning, eye development, oocyte development and limb chondrocyte differentiation (de Maximy *et al*. 1999). So far, three sprouty genes have been identified in the human and four in the mouse (Hacohen *et al*. 1998; de Maximy *et al*. 1999). Overexpression of mouse *sprouty 2* (*Spry2*) and mouse *sprouty 4* (*Spry4*) resulted in repression of limb development in chicken (Minowada *et al*. 1999), while ablation of *Spry2* expression in cultured embryonic mouse lungs led to an increase in lung branching morphogenesis (Tefft *et al*. 1999). The proteins of the Sprouty family show great homology with each other in the C-termini; their N-termini are different (Wong *et al*. 2001; Yigzaw *et al*. 2001).

Two human *SPYR4* gene sequences (*SPYR4C*, AF227517; and *SPYR4A*, AF227516) were earlier submitted to the GenBank database, but their identity was not verified by experimental data. Here we report on the expression and chromosome location of human *SPYR4*.

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Keywords. *SPRY4*; radioactive hybridization (RH); expression pattern.

Materials and methods

***Radiation hybrid (RH) mapping*:** To determine the location of the gene, we screened the Stanford G3 radiation hybrid (RH) panel using a *SPRY4*-specific primer pair for PCR. The PCR primers (5′-GAAAAGTGCTGGTTAGTTCCCGCCC-3′ and 5′-CATGAACCAGAAGGCTAGTGAGGC-3′) were designed to amplify the 3′ untranslated region shared by the two *SPYR4* sequences submitted earlier to GenBank. The data were submitted to RH Server (http://www-shgc.stanford.edu/RH/rhserverformnew.html) to see which SHGC-typed (Stanford Human Genome Center, Stanford, USA) markers on the G3 panel are closest to the gene. Then the SHGC-typed markers were placed on the SHGC G3 RH maps (http://www-shgc.stanford.edu/Mapping/rh/search.html). The most likely chromosome band location of the gene was deduced from the physical mapping information of flanking markers (Genome DataBase, GDB).

***Northern blot analysis*:** Multiple-tissue Northern blots (MTN blots) containing 2 μg of polyA^+ mRNA isolated from a variety of human tissues were purchased from Clontech. MTN blots were probed with labelled PCR product, which was obtained by the following procedure. The same primers used for RH analysis were designed to amplify the probe, using premade human foetal brain cDNA (Clontech) as template. PCR product was labelled with [$\alpha-^{32}$P]dCTP by random priming with the Promega labelling kit. Northern hybridization was performed according to the manufacturer's recommendations (Clontech).

***RT-PCR analysis*:** Multiple-tissue cDNA panels (MTC) I and II and human foetal tissue cDNA panels were purchased from Clontech. MTC-based reverse transcriptase PCR (RT-PCR) was performed according to the manufacturer's recommendations. Briefly, the same primers as those used for RH analysis and Advantage 2 DNA polymerase were used to detect expression of *SPYR4* in different tissues. The PCR product was used both for agarose gel examination and for sequence analysis.

Results

Chromosomal mapping of SPYR4

The chromosomal location of *SPYR4* was determined using the Stanford G3 radiation hybrid panel with a primer pair specific to the 3′ untranslated region of *SPYR4* and submitting the result (data vector: 10100000000000001000 00000001000000000000000010000001000000010010001 0000000000000000) to RH Server for maximum likelihood estimation. The expected 544-bp PCR product was amplified from nine out of the 83 hybrid cell lines in the panel. *SPYR4* was placed at 7 centirays (cR_3000) from SHGC-13178 (LOD_SCORE = 10.63), 18.0 cR_3000 from SHGC-111096 (LOD_ SCORE = 8.65) and 18.0 cR_3000 from SHGC-58162 (LOD_SCORE = 8.65). Available physical mapping information about flanking markers (GDB) indicated that *SPYR4* was located in the 5q32→q33 region (figure 1).

Expression pattern of SPYR4

Expression analysis of *SPYR4* cDNA was undertaken by Northern blot and RT-PCR analysis using multiple-tissue Northern blot and multiple-tissue cDNA panels. A probe for *SPYR4* hybridized to two different sized mRNAs from liver, skeletal muscle, heart, lung, kidney, spleen, placenta and small intestine. The larger mRNA is about 5.0 kb, the smaller one about 2.0 kb. No hybridization signal was seen for leukocytes, thymus, brain and colon. (figure 2).

RT-PCR analysis showed that the transcripts of *SPYR4* were seen in all the tested tissues with different intensities (figure 3).

Discussion

Sprouty was originally identified as an inhibitor of *Drosophila* FGF receptor signalling during tracheal development. Moreover, Sprouty acts as an antagonist of EGF as well as FGF signalling pathways in *Drosophila* (Kramer *et al.* 1999). Up to now, four mammalian *sprouty* genes (Spry1–Spry4) have been identified (Hacohen *et al.* 1998; de Maximy *et al.* 1999; Minowada *et al.* 1999; Reich *et al.* 1999). Sprouty proteins contain a cysteine-rich domain that is highly conserved in three human and four mouse homologues (Hacohen *et al.* 1998; de Maximy *et al.* 1999; Minowada *et al.* 1999; Tefft *et al.* 1999). Mouse homologues of *sprouty* (*Spry1*, *Spry2*, *Spry3*, *Spry4*)

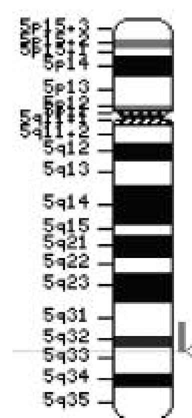

Figure 1. *SPYR4* location on chromosome 5 determined by radiation hybrid mapping.

are expressed in embryonic and adult tissues such as brain, heart, kidney, lung, limbs and skeletal muscle (de Maximy *et al.* 1999; Minowada *et al.* 1999; Tefft *et al.* 1999). *Spry4* is expressed in mouse embryo and developing lung (de Maximy *et al.* 1999), and *Spry4* expression inhibited branching and sprouting of small vessels (Lee *et al.* 2001). These studies indicate that Spry4 inhibits receptor tyrosine kinase signalling upstream of *Ras* (Lee *et al.* 2001). The observation of Sasaki and coworkers (2001) suggests that endogenous Spry2 and Spry4 are negative feedback regulators of growth-factor-mediated extracellular signal-related kinase (ERK) pathway.

Two human *sprouty 4* genes were submitted earlier to GenBank. *SPYR4C* with length of 7053 bp encodes a 106-residue protein. The location of the last 2625 bp (7053–4428) was identified as the human chromosome 11 contig Hs11_33417, while the location of the gene segment 2545–4428 was assigned to human chromosome 5 contig Hs_29448. Moreover, all the 5′ sequences that contain ORF seemed to be located in a gap in the available sequence. *SPRY4A* with length of 4934 bp encodes a 322-residue protein. The gene segments 3049–4932 and 159–210 were assigned to Hs_29448. Again, the fragment containing ORF was located to a gap. By screening the Stanford G3 RH panel using primers corresponding to the common region of *SPYR4C* and *SPYR4A*, we assigned *SPYR4* to chromosome 5q32.

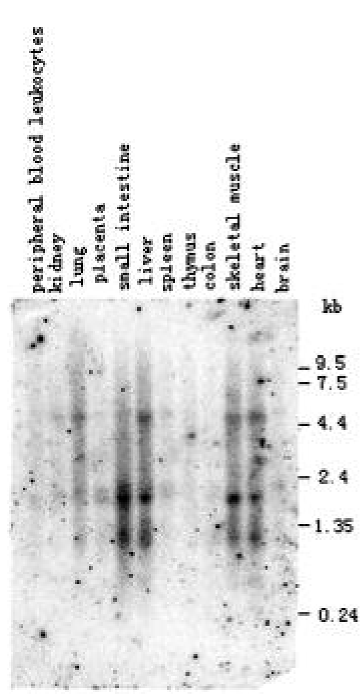

Figure 2. *SPYR4* Northern blot analysis. The RNA size marker positions are indicated on the right. Two mRNA transcripts can be detected, of approximately 5.0 and 2.0 kb, in multiple tissues. Note the high expression in liver, skeletal muscle, heart, small intestine and lung.

Northern blot analysis suggests that the transcript of *SPYR4* may be an mRNA of length about 5 kb. A band of about 2 kb also appears with a pattern similar to that of the 5-kb signal. We are not sure what it is. However, we did not detect any band of length greater than 7 kb. BLAST analysis against NCBI genome database revealed that *SPRY4C* contained sequence from both chromosome 11 and chromosome 5, so we thought the sequence of *SPRY4C* might be wrong.

BLAST analysis against the human dbEST database (http://www.ncbi.nlm.nih.gov/dbEST) shows that the common region of *SPYR4C* and *SPYR4A* is populated by many ESTs, while very few ESTs are known in the unique regions. We performed a 5′ RACE (rapid amplification of cDNA ends) and tried to clone the 5′ terminus of *SPYR4* with the 3′ specific primer corresponding to the common region. But we got neither the 5′ terminus of *SPYR4C* nor *SPYR4A* (data not shown).

Northern blots and MTC-based RT-PCR revealed that the transcript is present ubiquitously in human tissues. The low expression in leukocytes is easy to understand because leukocytes are not involved in branch development. We cannot give a reasonable explanation for the very low expression in pancreas.

In conclusion, our study has located *SPYR4* to chromosome 5q23 and revealed that the transcript of *SPYR4* is seen in multiple tissues at different levels.

Note: After we submitted this article for publication, it has come to our attention that a similar work has been pub-

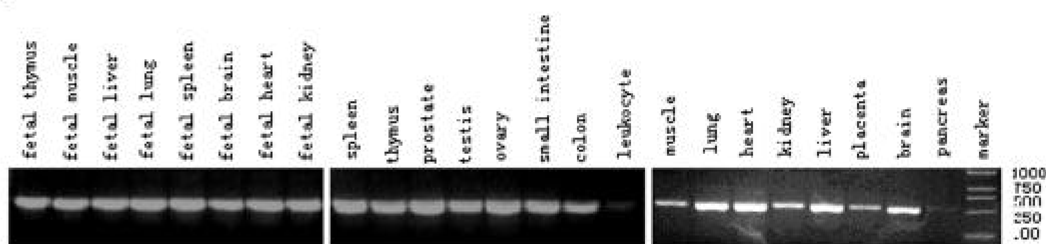

Figure 3. *SPYR4* RT-PCR analysis. *SPYR4* gene transcript is detected in all tested tissues with different intensities.

lished by Leeksma *et al*. (Leeksma O. C., Van Achterberg T. A., Tsumura Y., Toshima J., Eldering E., Kroes W. G., Mellink C., Spaargaren M., Mizuno K., Pannekoek H. and de Vries C. J. 2002 Human sprouty 4, a new ras antagonist on 5q31, interacts with the dual specificity kinase TESK1. *Eur. J. Biochem*. **269**, 2546–2556).

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References

de Maximy A. A., Nakatake Y., Moncada S., Itoh N., Thiery J. P. and Bellusci S. 1999 Cloning and expression pattern of a mouse homologue of *Drosophila sprouty* in the mouse embryo. *Mech. Dev*. **81**, 213–216.

Freeman M. 2000 Feedback control of intercellular signaling in development. *Nature* **408**, 313–319.

Hacohen N., Kramer S., Sutherland D., Hiromi Y. and Krasnow M. A. 1998 *Sprouty* encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airway. *Cell* **92**, 253–263.

Kramer S., Okabe M., Hacohen N., Krasnow M. A. and Hiromi Y. 1999 Sprouty: a common antagonist of FGF and EGF signaling pathways in *Drosophila*. *Development* **126**, 2515–2525.

Lee S. H., Schloss D. J., Jarvis L., Krasnow M. A. and Swain J. L. 2001 Inhibition of angiogenesis by a mouse Sprouty protein. *J. Biol. Chem*. **276**, 4128–4133.

Metzger R. J. and Krasnow M. A. 1999 Genetic control of branching morphogenesis. *Science* **284**, 1635–1639.

Minowada G., Jarvis L. A., Chi C. L., Neubüser 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.

Reich A., Sapir A. and Shilo B.-Z. 1999 Sprouty is a general inhibitor of receptor tyrosine kinase signaling. *Development* **126**, 4139–4147.

Sasaki A., Taketomi T., Wakioka T., Kato R. and Yoshimura A. 2001 Identification of a dominant negative mutant of Sprouty that potentiates fibroblast growth factor- but not epidermal growth factor-induced ERK activation. *J. Biol. Chem*. **276**, 36804–36808.

Tefft J. D., Lee M., Smith S., Leinwand M., Zhao J., Jr P. B., Crowe D. L. and Warburton D. 1999 Conserved function of mSpry-2, a murine homolog of *Drosophila sprouty*, which negatively modulates respiratory organogenesis. *Curr. Biol*. **9**, 219–222.

Wong E. S. M., Lim J., Low B. C., Chen Q. and Guy G. 2001 Evidence for direct interaction between sprouty and Cb1. *J. Biol. Chem*. **276**, 5866–5875.

Yigzaw Y., Cartin L., Pierre S., Scholich K. and Patel T. B. 2001 The C terminus of Sprouty is important for modulation of cellular migration and proliferation. *J. Biol. Chem*. **276**, 22742–22747.

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