Sprouty1 haploinsufficiency accelerates pheochromocytoma development in Pten+/- mice

Dear Editor,

Pheochromocytomas (PCCs) are rare catecholamine-secreting tumors arising from the chromaffin cells in the adrenal medulla. Closely related paragangliomas share developmental origin but arise extra-adrenally in the paraganglia that belongs to the sympathetic and parasympathetic ganglia. Most PCCs are sporadic; however, around 25–30% of them are associated with familial syndromes caused by germ line mutations of at least ten genes (Gimenez-Roqueplo et al. 2012). The low incidence of pheochromocytomas poses difficulties to the development of diagnostic or prognostic markers as well as effective therapies, and thus, the development of novel animal and cellular models for the study of the disease is needed. Sprouty (Spry) family of genes is composed of four members of feedback inhibitors of receptor tyrosine kinase signaling that specifically targets the MAPK pathway. As such, they have been proposed as tumor suppressors in several cancerous pathologies such as tumors of the prostate, thyroid, or liver (Masoumi-Moghaddam et al. 2014). Here, we present a novel mouse model of pheochromocytoma consisting of double-heterozygous mice for Pten and Sprouty1 (Spry1). These animals develop pheochromocytomas that appear at earlier onset and grow at a higher rate than those from Pten+/− mice.

In a previous report, we have found that mice bearing a Spry1-null mutation in the context of Pten haploinsufficiency developed thyroid hyperplasia at higher frequencies and earlier onset than Pten+/- littermates (Macià et al. 2014). While examining thyroid glands from these animals, we noticed that Pten+/-; Spry1 +/- mice also developed thyroid hyperplasia with a frequency comparable to that of Pten+/-; Spry1+/- mice at 3 months of age (M Vaquero, A Macià, C Anerillas, A Velasco, X Matias-Guiu, J Ribera and M Encinas, unpublished observations), indicating that deletion of a single Spry1 allele was enough to accelerate thyroid tumorigenesis in the context of Pten haploinsufficiency. We therefore looked for tumor formation in tissues from Pten+/-; Spry1+/- double-heterozygous mice in other tissues, including the adrenal gland, which expresses high levels of Spry1 by real-time PCR (M Vaquero, A Macià, C Anerillas, A Velasco, X Matias-Guiu, J Ribera and M Encinas, unpublished observations). Histological analysis of mice at 5 months of age revealed that roughly one third (9/28; 32%) of adrenal glands from double-heterozygous mice presented pheochromocytoma compared with 17% (3/18) of adrenals from Pten+/- mice (Fig. 1A and B). In these lesions, the alveolar or ‘nested’ pattern found on normal medullae was replaced by more densely packed sheets or cords of cells that compressed the adjacent cortical parenchyma (Fig. 1A).

Most of the remaining adrenal glands of double-heterozygous mice (15/28; 50%) developed hyperplastic nodules (Tischler et al. 2004) in their medullae (Fig. 1A and B), which were similar in morphology to pheochromocytomas but did not compress the adrenal cortex. In contrast, only four out of 18 glands (22%) from Pten+/- mice developed nodular hyperplasia at this age, being the remaining 60% normal. Overall, at five months of age, more than 80% of double-mutant mice compared with around 40% of Pten+/- mice showed neoplastic lesions of the adrenal medulla. Medullae from Spry1+/- mice were normal in all cases and at all ages analyzed (Fig. 1A). Tumors from both Pten+/- and double-heterozygous mice stained positive for tyrosine hydroxylase, demonstrating their chromaffin origin (Fig. 1C). We next conducted loss of heterozygosity analysis by PCR, using primers flanking a FRT site present in the knockout but not the wild-type allele (Basson et al. 2005). Capillary electrophoresis fragment analysis indicated that the wild-type allele was retained.
in ten out of ten pheochromocytomas from double-heterozygous mice analyzed (Fig. 1D). Moreover, immunoblot analysis of SPROUTY1 levels from pheochromocytomas using a specific antibody (D9V6P; Cell Signaling Technologies) clearly showed that expression of SPROUTY1 was not lost in five out of six
tumors from double-heterozygous mice (Fig. 1E). Interestingly, SPROUTY1 protein levels were reduced in pheochromocytomas from double-heterozygous mice when compared with Pten+/− mice, likely reflecting that Spry1 behaves as a dosage-sensitive gene. This is in agreement with our previous findings indicating that Spry1 also behaves as a dosage-sensitive gene during genitourinary development, as deletion of a single allele of Spry1 partially rescues renal agenesis caused by Ret mutation (Rozen et al. 2009). In conclusion, loss of a single allele of Spry1 in the context of Pten haploinsufficiency significantly (P=0.01, χ² test) accelerated the appearance of neoplastic lesions of the adrenal medulla. Immunocytochemistry and western blot analysis also indicated that PTEN expression was frequently lost in pheochromocytomas from both genotypes (M Vaquero, A Macià, C Anerillas, A Velasco, X Matias-Guiu, J Ribera and M Encinas, unpublished observations). By 9 months of age, virtually all double-heterozygous mice examined presented either pheochromocytoma (21/32; 66%) or nodular hyperplasia (9/32; 28%), whereas 14/30 (47%) and 12/30 (40%) of Pten+/− adrenal glands did (Fig. 2C). These differences were not statistically significant (P=0.29, χ² test); however, adrenal glands from double-heterozygous mice were on average almost four times bigger than those from Pten+/− mice (Fig. 2A and B). Thus, adrenal glands from Spry1+/− mice had a mean volume of 0.79±0.19 mm³ (mean ± standard error) compared with 2.64±0.51 mm³ (Pten+/−) and 8.28±1.77 mm³ (Pten+/−; Spry1+/−). Differences in adrenal size between Pten+/− and Pten+/−; Spry1+/− mice were highly significant (P=0.0001,
Mann–Whitney test). The number of mitotic figures per 10 high power fields was also significantly higher in pheochromocytoma from double-heterozygous mice than those from Pten+/- mice (P=0.033, Mann–Whitney test, Fig. 2D). Of 14 Pten+/- pheochromocytomas, five (36%) presented more than three mitoses per 10 high power field, whereas 15 out of 21 (71%) Pten+/-; Spry1+/- did. These differences presented a trend toward but did not reach statistical significance (P=0.07, \( \chi^2 \) test).

Finally, tyrosine hydroxylase or synaptophysin staining revealed that larger glands from double-heterozygous mice but not from Pten+/- mice often presented chromaffin cells invading the adrenal cortex or breaking through the adrenal capsule (Fig. 2E). Although tumor-associated criteria reliably predicting malignancy in pheochromocytoma are not available, tumor size, local invasion, or mitotic index have been proposed as signs of malignancy by several authors (Mete et al. 2014). In conclusion, our data demonstrate that loss of a single allele of Spry1 accelerates both the onset of pheochromocytoma formation and the growth rate of these tumors. We currently do not know by which mechanism Spry1 restrains growth of pheochromocytoma cells; however, several possibilities deserve future attention. Transcriptomic analysis of human pheochromocytomas and paragangliomas reveals two different clusters characterized by distinct expression signatures (Gimenez-Roqueplo et al. 2012). Cluster 1 (comprising mutations of SDHx, FH, VHL, or HIF2A (<i>EPAS1</i>)) presents a pseudo-hypoxia signature characterized by a switch from oxidative phosphorylation to aerobic glycolysis (Warburg effect), whereas cluster 2 (mutations of <i>RET</i>, <i>NF1</i>, <i>TMEM127</i>, or <i>MAX</i> reflects aberrant activation of the MAPK and mTOR pathways. One mechanism worth exploring would be ectopic activation of the MAPK kinase pathway upon Spry1 loss. Interestingly, Spry1 and Spry2 antagonize Ret signaling during the development of renal and enteric nervous system, via inhibition of the MAPK (and the PI3-K) pathways (Masoumi-Moghaddam et al. 2014). However, our previous work points to a role of Spry1 in inducing cellular senescence as a mechanism of tumor suppression in the thyroid gland (Macià et al. 2012, 2014). Similarly, induction of cellular senescence could account for the protective effects of Spry1 in pheochromocytoma development. In line with this hypothesis, You et al. (2002) have demonstrated that pheochromocytoma incidence in Pten+/- mice dramatically increases upon concomitant deletion of the Ink4a/Arf locus, a master regulator of cellular senescence. Recent studies demonstrate that escape from senescence is accompanied by a metabolic switch from oxidative phosphorylation to aerobic glycolysis (Kaplon et al. 2013), much like the pseudo-hypoxic response found in cluster 1. In any case, further studies are required to determine whether Spry1 restricts pheochromocytoma growth by inhibiting the MAPK pathway, by inducing cellular senescence, or a combination of both.

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Declaration of interest
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References
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