The *NF1* gene: a frequent mutational target in sporadic pheochromocytomas and beyond

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Abstract

Patients suffering from the neurofibromatosis type 1 syndrome, which is caused by germline mutations in the *NF1* gene, have a tiny but not negligible risk of developing pheochromocytomas. It is, therefore, of interest that the *NF1* gene has recently been revealed to carry somatic, inactivating mutations in a total of 35 (21.7%) of 161 sporadic pheochromocytomas in two independent tumor series. A majority of the tumors in both studies displayed loss of heterozygosity at the *NF1* locus and a low *NF1* mRNA expression. In view of previous findings that many sporadic pheochromocytomas cluster with neurofibromatosis type 1 syndrome-associated pheochromocytomas instead of forming clusters of their own, *NF1* inactivation appears to be an important step in the pathogenesis of a large number of sporadic pheochromocytomas. A literature and public mutation database review has revealed that pheochromocytomas are among those human neoplasms in which somatic *NF1* alterations are most frequent.

Key Words

- ▶ *NF1*
- ▶ pheochromocytomas
- ▶ molecular genetics
- ▶ somatic mutations
- ▶ sporadic tumors

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The neurofibromatosis type 1 syndrome, caused by germline mutations in the *NF1* gene, is a multisystem tumor predisposition disorder associated with neurologic, cutaneous, and orthopedic manifestations. Only a small fraction, about 1% (range 0.1–5.7%), of patients with neurofibromatosis type 1 develop pheochromocytomas (Walther *et al.* 1999). Hereditary pheochromocytomas can also be observed in von Hippel–Lindau disease (10–26%) and multiple endocrine neoplasia type 2 (about 50%), as well as in patients with mutations in the succinate dehydrogenase (*SDH*) genes (having a wide range depending on the gene affected) and a few additional more recently discovered susceptibility genes (reviewed in Welander *et al.* (2011)). Until recently, somatic mutations in any of the genes involved in hereditary

pheochromocytoma have been thought to be rare in the more common sporadic form of the tumor, as has repeatedly been reported for the *RET*, *VHL*, and *SDHx* genes (Burnichon *et al.* 2011). While ~15% of all the pheochromocytomas or paragangliomas are thought to be associated with germline *SDHx* mutations (Gill *et al.* 2010, Welander *et al.* 2011), no single *SDHx* gene is affected in more than 10% of the cases at the most. Genome-wide expression studies have revealed that pheochromocytomas and paragangliomas cluster into two distinct groups based on their transcription profile: *VHL*- and *SDHx*-related tumors display a similar gene expression profile associated with hypoxia and angiogenesis, whereas *RET*- and *NF1*-related tumors express genes linked to an activation of kinase signaling pathways (Eisenhofer *et al.*

2004, Dahia *et al.* 2005). Interestingly, sporadic pheochromocytomas cluster into either of the two distinct groups instead of forming clusters of their own.

Two independent studies (Burnichon *et al.* 2012a, Welander *et al.* 2012) have recently revealed that the *NF1* gene is the most frequent target of somatic, truncating mutations in sporadic pheochromocytomas known to date. This suggests one cause for the observation that subgroups of sporadic pheochromocytomas share a common transcriptional profile with hereditary *NF1*/*RET*-related tumors. The study carried out by Burnichon *et al.* (2012a,b) reported mutations in 25 of the 61 (41%) of investigated tumors. Here, the authors pre-selected pheochromocytomas by genome-wide expression cluster analysis and carried out *NF1* mutation analysis on a subset of those displaying a *NF1*/*RET*-like gene expression pattern, giving a mutation frequency in their entire cohort of 119 sporadic pheochromocytomas of 21.8%. This is in agreement with the study carried out by Welander *et al.* (2012), where 10 of the 42 (23.8%) unselected sporadic pheochromocytomas exhibited somatic *NF1* mutations. Thus, current data suggest that roughly one-fifth to one-fourth of the sporadic

pheochromocytomas harbor somatic *NF1* mutations. In agreement with the classic tumor suppressor gene model, a majority of the tumors in both studies displayed loss of heterozygosity (LOH) at the *NF1* locus and a low *NF1* mRNA expression. In addition, a previous study has reported LOH at the *NF1* locus in a substantial proportion of sporadic pheochromocytomas (Sandgren *et al.* 2010), while another early study has reported a lack of neurofibromin protein expression in one of the four sporadic pheochromocytomas (Gutmann *et al.* 1995). It is noteworthy that high plasma levels of catecholamines emerged as common features in affected patients of the *NF1*-mutated pheochromocytomas (Welander *et al.* 2012). However, the biochemical data were non-centralized and incomplete in this study, limiting the significance of this finding. Still, the observation of high catecholamine levels in patients with neurofibromatosis type 1 and pheochromocytomas and the finding that PNMT, the enzyme responsible for the conversion of norepinephrine to epinephrine, is normally expressed in *NF1* and *RET* tumors in contrast to the other hereditary pheochromocytomas would support this finding (Eisenhofer *et al.* 2011a,b, Burnichon *et al.* 2012b).

Figure 1

Frequency of somatic *NF1* gene alterations in different sporadic human neoplasms. MPNST, malignant peripheral nerve sheath tumor; AML, acute myelogenous leukemia.

Table 1 NF1 mutation distribution in human neoplasms according to the COSMIC public mutation database (<http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=NF1#dist>) as of May 8th 2013

Primary tissue	Substitution nonsense	Substitution missense	Substitution synonymous	Insertion inframe	Insertion frameshift	Deletion inframe	Deletion frameshift	Complex	Other	Unique mutated samples	Total unique samples	Percentage of mutated samples
Adrenal gland	1	1					1		8	11	2	0.00
Autonomic ganglia											226	4.87
Biliary tract											16	0.00
Bone											50	0.00
Breast	1	6			1		2		1	10	550	1.82
CNS	18	13	3		2	1	19		11	58	1104	5.25
Cervix	1	2	2							3	26	11.54
Endometrium	2	7	2						2	12	238	5.04
Eye											92	0.00
Gastrointestinal tract ^a											1	0.00
Hematopoietic/ lymphoid	17	19	8		7		3		7	51	1117	4.57
Kidney	1	6	1						1	9	535	1.68
Large intestine	3	19	1		2		4	1	11	34	853	3.99
Liver		1								1	81	1.23
Lung	19	57	13		1		4		17	101	1262	8.00
Meninges											10	0.00
Esophagus											46	0.00
Ovary	4	9	2		1		3		1	19	724	2.62
Pancreas		3	1							4	389	1.03
Placenta											2	0.00
Pleura											6	0.00
Prostate		2								2	399	0.50
Salivary gland											2	0.00
Skin	5	12	2				3		4	23	222	10.36
Small intestine											1	0.00
Soft tissue	62	18			17	4	82	5	61	248	744	33.33
Stomach	1								1	2	60	3.33
Testis											4	0.00
Thyroid	1									1	11	9.09
Upper aerodigestive tract	1	3	1							5	138	3.62
Urinary tract									1	9	61	14.75
Vulva	1	7									3	0.00
Total	138	185	36	0	31	5	121	6	126	603	9048	6.66

^aSite indeterminate.

Inactivation of neurofibromin leads to the activation of the RAS/RAF/MEK/ERK pathway (Ballester *et al.* 1990, Martin *et al.* 1990). The importance of this signaling pathway in sporadic pheochromocytoma development has been very recently confirmed when somatic *H-RAS* mutations were reported in these tumors (Crona *et al.* 2013), and studies of additional factors in the pathway may thus be warranted.

Somatic *NF1* gene mutations have in recent years also been detected in other forms of sporadic neoplasms, including glioblastomas (The Cancer Genome Atlas Research Network 2008), malignant peripheral nerve sheath tumors (MPNSTs; Bottillo *et al.* 2009), acute myelogenous leukemia (Parkin *et al.* 2010), neuroblastomas (Holzel *et al.* 2010), lung adenocarcinomas (Ding *et al.* 2008), and ovarian carcinomas (The Cancer Genome Atlas Research Network 2011), indicating that the *NF1* gene may represent a significant mutational target in both neural- and non-neural-derived sporadic tumors (Fig. 1). The public mutation database COSMIC (<http://cancer.sanger.ac.uk/cosmic/gene/analysis?ln=NF1#dist>) has revealed that *NF1* is a target with a varying mutation frequency in a large number of different human neoplasms (Table 1). Apart from MPNSTs and the large group of soft-tissue tumors (including blood vessels, fat, and fibrous tissue, as well as smooth and striated muscle), sporadic pheochromocytomas appear to be the most frequent target. Interestingly, LOH at the *NF1* locus, not associated with the mutation, appears in a proportion of all the neoplasms studied (Fig. 1). Concerning pheochromocytomas, *NF1* mutations are sometimes observed in samples without LOH (Burnichon *et al.* 2012a). This opens up a discussion of other potential factors affecting *NF1* expression (hypermethylation has been excluded in some but not all positions in the promoter (Welander *et al.* 2012)) or possibly haploinsufficient behavior.

Many, but not all, of the neoplasms with somatic *NF1* mutations have also been reported in patients with neurofibromatosis type 1 syndrome. Thus, the role of *NF1* as a tumor suppressor in other malignancies not associated with neurofibromatosis type 1 syndrome warrants further studies.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the commentary reported.

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