

Figures and Tables from de Pontual et al. 2007

Table 1.

Presentation of a series of 13 NB cell lines and 18 tumours for which PHOX2B expression data were analysed on Affymetrix HG U133 Plus 2.0 arrays (Affy Exp) and/or RT-PCR. Mutations, LOH and methylation analyses are presented as well as prognostic factors (stage, loss of 1p36, N-MYC amplification; A, Amplified; NA, Non Amplified)

		<i>PHOX2B</i>								
		Affy Exp	RT- PCR	Mutation	LOH	Methylation	Stage INSS	Loss of 1p36	N- MYC	
Cell lines	CLB-BAR	3679	+	–	–	–	4	+	A	
	CLB-GA	6956	+	–	–	–	4	+	NA	
	CLB-GE	5725	+	–	–	–	4	+	A	
	CLB-MA	7820	+	–	–	–	4	+	A	
	CLB-PE	387	+	–	–	–	2	–	A	
	GIMEN	6	–	–	–	+	4	+	NA	
	IMR32	8839	+	–	–	–	2	+	A	
	KCNR	5181	+	–	–	–	4	+	A	
	SJNB12	11010	+	–	–	–	3A	+	NA	
	SKNAS	2169	+	–	–	–	4	+	NA	
	SKNSH	23	–	721–740del20nt	–	–	4	–	NA	
	SKNBE	6	–	–	–	+	4	+	A	
	TR14	2534	+	–	–	–	3	+	A	
Tumours	NBAF15	6775	ND	–	–	–	2B	–	NA	
	NBAF21	136	–	–	–	+	3	–	NA	
	NBAF26	8259	+	–	–	–	4S	–	NA	
	NBAF38	23	–	–	+	–	2A	–	NA	

		<i>PHOX2B</i>							
		Affy Exp	RT-PCR	Mutation	LOH	Methylation	Stage INSS	Loss of 1p36	N-MYC
	NBAF39	6293	ND	–	–	–	2B	–	NA
	NBAF40	7462	ND	–	+	–	4S	–	NA
	NBAF42	7557	ND	–	–	–	4	–	NA
	NBAF43	3415	+	–	–	–	2B		
	NBAF45	7089	+	–	–	–	1	–	NA
	NBAF46	1394	+	–	–	–	2A	–	NA
	NBAF5	1053	ND	299G > T(R100L)	–	–	1	–	NA
	NBAF50	7811	ND	–	–	–	2B	–	NA
	NBAF54	5166	ND	–	+	–	4S	–	NA
	NBAF56	12059	ND	–	–	–	4S	+	NA
	NBAF59	10706	+	–	+	–	2A	–	NA
	NBAF61	10684	ND	–	+	–	4S	–	NA
	NBAF64	5349	ND	–	–	–	4	+	NA
	NB10	ND	–	–	–	+	2B	–	NA

Fig. 1. (a) *PHOX2B* mRNA in NB cell lines. *PHOX2B* mRNA is detected in 10/13 cell lines as in human adrenal gland medulla (MSR) and non-transformed human neural crest cells (NC). No expression could be observed in three NB cell lines; SKNSH, SKNBE and GIMEN. (b) Bisulfite sequencing analysis of the methylation status of the 12 CpG from the start site. One microgram of tumour DNA was denatured by sodium hydroxide and modified by sodium bisulfite treatment, which converts unmethylated cytosines to uracil. Bisulfite treated DNA was amplified using specific primers (added in methods). *PHOX2B* promotor is 100% methylated in SKNBE and GIMEN but not in other cell lines. All the 12 CpG dinucleotide are methylated in two primary tumours NBAF21 and NB10. All ten clones showed identical methylation in each sample. •methylated cytosine ○ non-methylated cytosine.

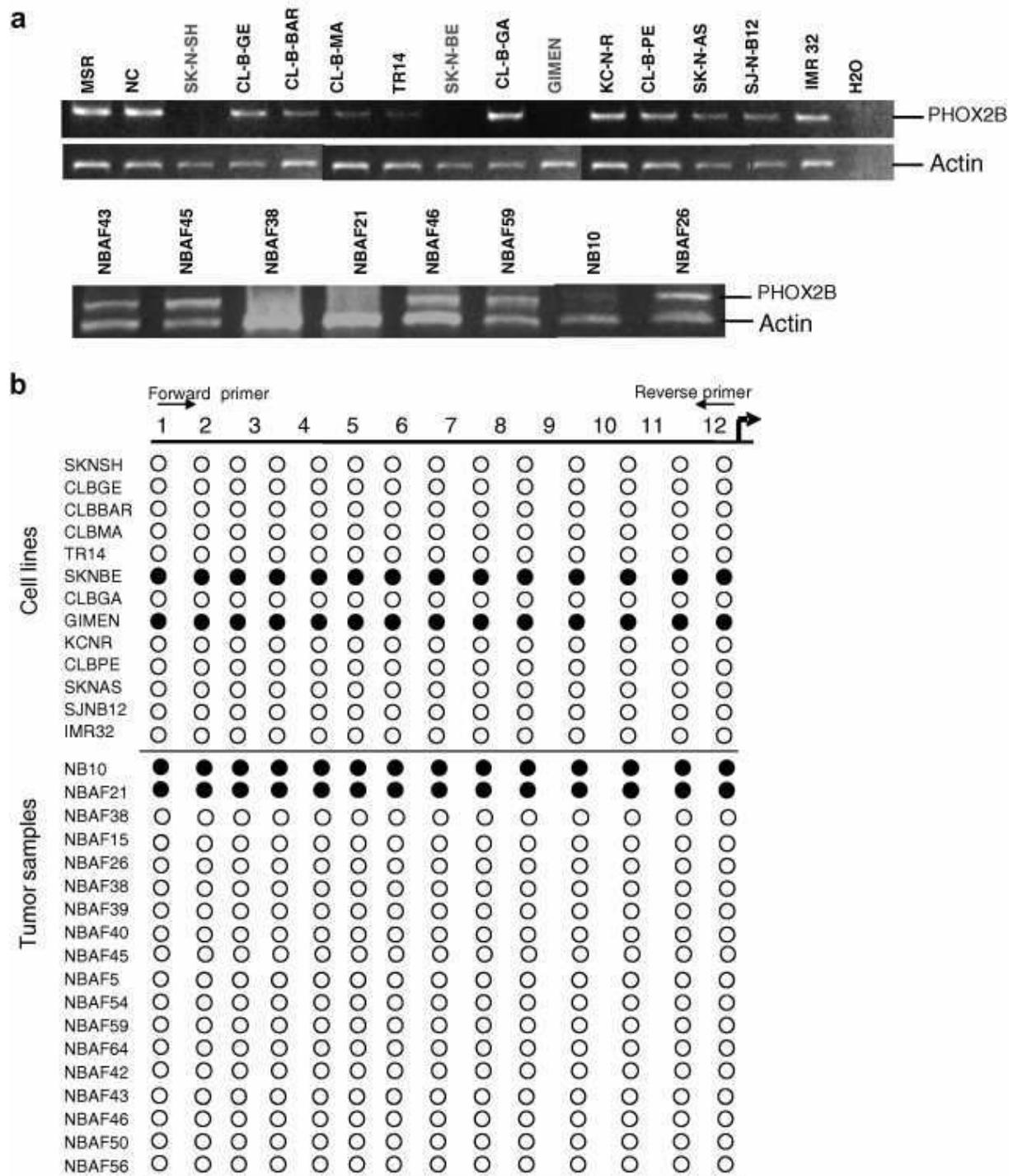


Fig. 2. (a) RT-PCR: Demethylation of the *PHOX2B* promoter CpG islands restores expression in *PHOX2B*-negative NB cell lines, SKNBE and GIMEN. SKNBE, GIMEN and SKNSH were treated (+) or not treated (-) with 1  $\mu$ mol/l 5'-aza-2'-deoxycytidine for three days. (b) Methyl sequencing results of *PHOX2B* promotor before (1) and after treatment (2) by 5-azacytidine.

