

Letter to the Editor

Low levels of *BNIP3* promoter hypermethylation in invasive breast cancer

Dear Sir,

Bcl-2/adenovirus E1B 19 kDa-interacting protein 3 (*BNIP3*) is a pro-apoptotic member of the Bcl-2 family induced under hypoxia [3,5,6]. Low or absent expression has been described in several human tumors, resulting in poor prognosis [1]. We previously reported that *BNIP3* expression is lost in a significant portion of invasive breast cancers, which was correlated with poor prognostic features such as positive lymph node status and high proliferation [9]. Since DNA promoter hypermethylation (“methylation”) is a common mechanism to silence gene expression, contributing to tumor-progression and invasion, we further studied the relation between *BNIP3* methylation and gene expression by RNA *in situ* hybridization (ISH) in invasive breast cancer.

In 40 cases of invasive breast cancer, *BNIP3* methylation was studied by Methylation Specific Multiplex Ligation-dependent Probe Amplification [2,4,8, 11] with 2 *BNIP3* probes (157 and 291) located in the CpG island harboring the *BNIP3* promoter. The 157 probe is located 50 nucleotides before exon 1, while probe 291 is located in exon 1. Both probes target sequences located in the *BNIP3* promoter that is located within a 1700-bp CpG island, which spans from –1162 to +538 bp of the transcription start site and contains the first exon of the *BNIP3* gene. This promoter area has been reported to be methylated in pancreatic cancer [12] and in gastric and colorectal cancer [10]. Data on *BNIP3* mRNA *in situ* hybridization were derived from a previous study [9].

BNIP3 methylation levels in 8 normal tissue samples was on average 0.08. *BNIP3* methylation was overall low (between 0.3 and 15% for probe 157 and between 0 and 31% for probe 291), while other genes included in this assay (*CCND2*, *RASSF1*, *GSTP1*, *HIN1*) showed methylation percentages up to 100% (means 24–51%) in the same tumors. Cases that were negative ($N = 4$) in *BNIP3* RNA ISH showed higher *BNIP3*

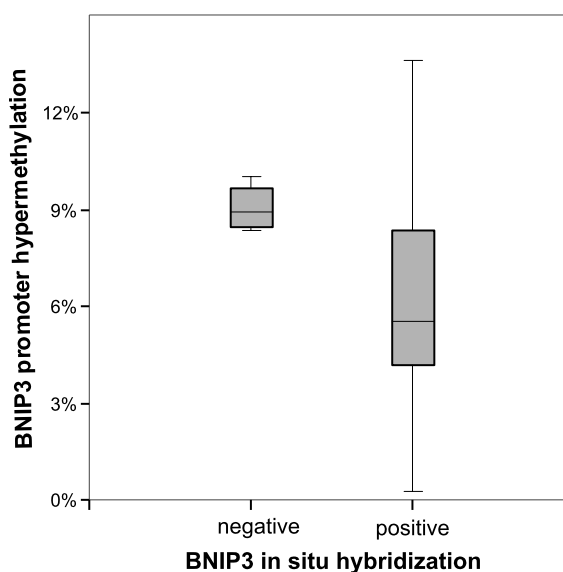


Fig. 1. Relation between *BNIP3* promoter hypermethylation (probe 157) and *BNIP3* RNA expression by *in situ* hybridization (4 cases were negative and 36 positive).

methylation ($p = 0.047$) for probe 157 than those positive by RNA ISH ($N = 36$) (see Fig. 1), but in none of the ISH negative tumors the methylation levels exceeded 10%. This correlation therefore needs to be interpreted with care. *BNIP3* methylation levels as detected by probe 291 were not significantly different ($p = 0.83$) between tumors with and without *BNIP3* expression.

In conclusion, *BNIP3* shows low levels of promoter hypermethylation in invasive breast cancer, mostly lower than the proposed 15% threshold that supposedly discriminates “true” from background methylation [7]. Although *BNIP3* methylation detected with probe 157 is associated with lower *BNIP3* expression at the RNA level, its low level suggests that methylation does not seem to be a major explanation for the differential *BNIP3* expression in invasive breast cancer previously described [9].

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