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Deletion of the *inhibitor of growth 4 (ING4)* tumor suppressor gene is prevalent in human epidermal growth factor 2 (HER2)-positive breast cancer☆

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Summary

Inhibitor of growth 4 (ING4) is a candidate tumor suppressor gene that was shown to be deleted in 10% to 20% of breast cancers by array comparative genome hybridization analysis. We developed fluorescent in situ hybridization to detect the *ING4* gene directly in the tissue samples on tumor tissue microarrays. We evaluated the *ING4* gene status in 1033 breast cancer tissue samples and observed that *ING4* was deleted in 16.5% (170/1033) of all breast cancers. *ING4* deletion was significantly associated with Her2 overexpression: of the tumors with *ING4* deletion, 23.8% (39/164) were human epidermal growth factor 2 (HER2) positive, as compared with 14.1% (115/814) of the tumors without *ING4* deletion ($P = .002$). In addition, the tumors with *ING4* deletion were more likely to belong to the HER2 molecular subtype (estrogen receptor negative/progesterone receptor negative/human epidermal growth factor positive) of breast cancer, compared with the other subtypes (28.4% HER2 versus 15.7% all, $P = .002$). *ING4* deletion did not affect survival outcome of all patients with breast cancer ($P = .797$) or of the patients with HER2-positive tumors ($P = .792$). We conclude that *ING4* deletion in breast cancer is relatively common, as 1 in 6 breast cancer harbors *ING4* deletion. Furthermore, *ING4* deletion is more prevalent in HER2-positive tumors, suggesting a functional antagonistic relationship between the *ING4* tumor suppressor and the HER2 oncogene. These results sustain the view that *ING4* is a tumor suppressor in breast cancer and suggest that *ING4* deletion may contribute to the pathogenesis of HER2-positive breast cancer.

Keywords

ING4; Tumor suppressor gene; Breast cancer; FISH; HER2/neu

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1. Introduction

ING4 is a member of the inhibitor of growth (ING) tumor suppressor family (ING1-5) and has been shown to play a role in cancer-related cellular processes, including cell proliferation, apoptosis, contact inhibition, tumor angiogenesis, DNA damage response, cell migration, and hypoxia [1–7]. The *ING4* gene is mapped to 12p13 in the short arm of chromosome 12. Loss of heterozygosity at 12p13 has been reported in cancers such as hematologic malignancies, ovarian cancer, and prostate cancer [8–10]. In addition, loss of heterozygosity and single-locus deletion of *ING4* have been found in head and neck squamous cell carcinoma and breast cancer, respectively [3,11]. Low levels of *ING4* expression have been correlated with high-grade tumors and poor patient outcome in malignant neoplasias, including glioma, melanoma, gastric adenocarcinoma, and hepatocellular carcinoma [4,12–14]. Moreover, inactivating point mutations of *ING4* have been found in several cancer cell lines and glioma [3,15]. Thus, *ING4* appears to be disabled by various mechanisms in cancer and may play a role as a tumor suppressor in various cancers that arise from diverse tissue types.

In a previous study, *ING4* deletion has been estimated to occur in 10% to 20% of breast cancers by array comparative genomics hybridization (aCGH) analysis using 2 DNA probes flanking the *ING4* gene [3]. In this study, we used fluorescent in situ hybridization (FISH) using a single bacterial artificial chromosome (BAC) clone that contains the *ING4* gene. We evaluated *ING4* deletion in more than 1500 breast cancer specimens using tissue microarray (TMA) and correlated *ING4* deletion with clinicopathologic parameters in breast cancer.

2. Materials and methods

2.1. Breast cancer TMA

TMAs contained 2020 tumor breast cancer tissue punches from 1579 independent formalin-fixed and paraffin-embedded tumor samples collected from patients with breast cancer diagnosed between the years 1985 and 2007 at the Institute for Pathology, University Hospital of Basel, and the Institute of Viollier in Basel, Switzerland. Of 2020 tissue spots on TMAs, 1566 tissue spots came from the same tumor samples used in the TMA studies previously described [16,19]. The median age of patients was 63 years ranging from 27 to 101 years. The mean follow-up time was 80.8 months ranging from 1 to 263 months. Raw patient survival data were obtained from the Cancer Registry of Basel or from the patients' attending physicians. Tumor data regarding histologic subtype, TNM classification, Bloom-Richardson-Elston-Ellis (BRE) grade, and diameter were obtained from pathology reports. Tissue samples and data were used according to the ethical standards of the University Hospital of Basel, Switzerland. TMAs were constructed as described previously [16]. In brief, tissue cylinders with a diameter of 0.6 mm were punched out from the "donor" tumor tissue blocks and transferred into a "recipient" paraffin block using a semiautomated tissue arrayer (Institute for Pathology, University Hospital of Basel, Basel, Switzerland). Each TMA contained a number of tumor punch spots ranging from 159 to 522. Three hundred thirty-six "double tissue spots" were included on TMAs from 168 samples by obtaining tissue punches from the tumor center and periphery.

2.2. Fluorescent labeling of the DNA probe

The BAC clone, RP11-433J6, was purchased from the Children's Hospital Oakland Research Institute (Oakland, CA). BAC DNA was purified from *Escherichia coli* using a plasmid purification kit (Qiagen, Valencia, CA). Eight hundred eighty nanograms of BAC DNA was digested with *Alu*I restriction enzyme (Invitrogen, Carlsbad, CA). *Alu*I-digested BAC DNA was labeled with Cy3-dUTP (GE Healthcare USA, Piscataway, NJ) using

BioPrime Array CGH kit (Invitrogen) for 2 hours at 37°C. Cy3-labeled BAC DNA (BAC-*ING4*) was purified using Vivaspin column (Sartorius Biolab, Göttingen, Germany). spectrum green–labeled chromosome 12 centromere probe (CEP12) was purchased from Vysis (Downers Grove, IL).

2.3. Fluorescent in situ hybridization

Metaphase spread of T47D cells was prepared using a standard method. In brief, cells were grown to 80% confluent and treated with colcemid (GIBCO, Grand Island, NY) at 10 μL/mL for 2 hours. Cells were harvested and incubated in prewarmed hypotonic solution (0.2% KCl, 0.2% sodium citrate) at 37°C for 7 minutes. Cells were washed, fixed in cold methanol/acetic acid (3:1 vol/vol), and dropped on a slide. Lymphocyte metaphase spread was purchased from Vysis. Paraffin-embedded normal breast tissue sections were obtained from the Institute for Pathology, University Hospital of Basel, Switzerland. Five-micrometer tissue and TMA sections were deparaffinized and pretreated with a paraffin pretreatment kit (Vysis).

Fluorescent-labeled DNA probes were hybridized to metaphase spread and tissue sections on slides at 37°C overnight. The slides were washed with wash solutions (Vysis; 0.4X saline-sodium citrate [SSC], 0.3% Nonidet P40, pH 7–7.5) and counterstained with 4′,6-diaminidino-2-phenylindole (DAPI) (Vysis) before mounting. The slides were visualized with a Zeiss Axiophot 2 epifluorescence microscope (Zeiss, Jena, Germany) using filter sets for DAPI, spectrum orange, spectrum green and DAPI/spectrum green/spectrum orange filter (Abbott Molecular, Abbott Park, IL). The FISH probe signals were counted in 10 nonoverlapping nuclei per tissue spot on TMA, and the BAC-*ING4*/CEP12 ratio was calculated. We used the ratio less than 0.8 to define a gene deletion, as described previously [17,18].

2.4. Immunohistochemistry

Immunochemical staining of TMA sections was performed using iView DAB Detection Kit (Ventana, Tucson, AZ). Antibodies used were anti–estrogen receptor α (anti-ER) monoclonal (1:40; Novocastra, Newcastle, UK), anti–progesterone receptor (anti-PR) monoclonal (1:100; Novocastra), and anti–HER2/neu polyclonal (no dilution; Ventana). The staining intensity of ER, PR, and HER2 was scored as described previously [19]. In brief, tumors were considered positive for ER or PR for nuclear staining in more than 10% of tumor cells, with an intensity score between 1 and 3. HER2 expression was scored as 0 for no staining, 1+ for faint and partial membranous staining, 2+ for weak complete staining of the membrane in more than 10% of tumor cells, and 3+ for intense complete staining of the membrane in more than 10% of tumor cells. All slides were scored manually by at least one pathologist (C.T. or E.K.).

2.5. Statistical analysis

Relationship between clinicopathologic features and *ING4* deletion in tumors was analyzed using Pearson χ^2 test. Wilcoxon rank sum test was used for the analysis of age and tumor size distribution. Kaplan-Meier method and log-rank test were used to assess survival time differences in univariate analysis and in subgroup analysis. All analyses were carried out using SAS V9.1 (SAS Institute, Cary, NC). The Bonferroni correction was used to adjust the level of significance for multiple comparisons, and *P* values less than .004 were considered statistically significant.

3. Results

3.1. FISH detection of the *ING4* gene

In a previous study, *ING4* was shown to be deleted in 10% to 20% of breast cancers, suggesting a tumor-suppressive role of *ING4* in breast cancer [3]. The deletion was estimated by aCGH analysis using 2 BAC probes flanking the *ING4* gene [3]. In this study, we used FISH using a single BAC clone that contains the *ING4* gene to determine the prevalence of *ING4* deletion in breast cancer. The *ING4* gene maps to the short arm of chromosome 12 and is located 6.7 mb from the telomere (www.ncbi.nlm.nih.gov). We chose BAC RP11-433J6 to detect the *ING4* gene (www.genome.ucsc.edu). The BAC is 176 kb in size and contains the *ING4* gene that spans 12.8 kb (Fig. 1A). First, we used 2-color FISH to determine the chromosomal location of the BAC on a metaphase spread of normal lymphocytes, using Cy3-labeled BAC (red) and spectrum green–labeled chromosome 12 centromere probe (green). We observed that Cy3-labeled BAC was hybridized to the short arm of chromosome 12, verifying the chromosomal location of the BAC (data not shown). We refer to the fluorescent-labeled BAC RP11-433J6 and chromosome 12 centromere probe as BAC-*ING4* and CEP12, respectively.

Next, we hybridized BAC-*ING4* to a metaphase spread of T47D breast cancer cells. T47D breast cancer cells were characterized as hypotriploid and contain 3 copies of chromosome 12 (www.ATCC.org). In a previous study, it was determined by aCGH that at least 2 copies of the *ING4* gene locus were deleted in T47D cells [3]. Using FISH, we detected 3 green signals of CEP12, indicating that T47D cells contain 3 copies of chromosome 12 (Fig. 1B). In contrast, only one red signal of BAC-*ING4* was detected (Fig. 1B). These results confirmed that T47D breast cancer cells contain 3 copies of chromosome 12 with 2 copies of the *ING4* gene deleted, retaining only 1 copy of the gene.

To evaluate the FISH probes on interphase chromosomes, we hybridized CEP12 and BAC-*ING4* to a 5-μm section of paraffin-embedded normal breast tissue. We detected 1 or 2 CEP12 signals and 1 or 2 BAC-*ING4* signals per nucleus. After counting 100 nuclei, the average ratio between BAC-*ING4* and CEP12 (BAC-*ING4*/CEP12) was approximately 1 (data not shown).

3.2. *ING4* deletion in breast tumor tissue samples on TMAs

We next hybridized CEP12 and BAC-*ING4* to TMAs containing breast cancer tissues. The probe signals were counted in 10 tumor cell nuclei per tissue spot. To avoid misinterpretation due to technical variability, we only counted the tissue areas that showed at least 1 signal for either BAC-*ING4* or CEP12. In addition, all TMA sections were hybridized twice to clarify uneven or insufficient hybridization of the probes. After excluding ambiguous and discrepant FISH scores, 1033 tissue spots (51.1%) among 2020 tissue spots were used for data evaluation.

We found that the BAC-*ING4*/CEP12 ratio ranged from 0.28 to 2.18 between the tissue samples on TMAs. A tumor with an overall ratio of 1 showed 2 BAC-*ING4* and 2 CEP12 signals per nucleus (Fig. 2A), indicating no deletion of *ING4*. A tumor that contained 0 to 3 BAC-*ING4* signals and 3 to 7 CEP12 signals per nucleus resulted in an overall ratio of 0.38 (Fig. 2B), showing underrepresentation of the *ING4* gene copy number compared with the number of chromosome 12 centromeres, thus indicating deletion of *ING4*. Another example of tumor with *ING4* deletion is shown in Fig. 2C, with 0 to 2 BAC-*ING4* signals and 2 to 7 CEP12 signals, resulting in an overall BAC-*ING4*/CEP12 ratio of 0.55 (Fig. 2C).

We used a ratio of 0.8 as a gene deletion reference point, as described previously [17,18]. We detected 170 tumor tissues with the BAC-*ING4*/CEP12 ratio of 0.8 or less, which made

up 16.5% (170/1033) of all breast tumors. We concluded that *ING4* is deleted in 16.5% of breast cancer. This indicated that *ING4* deletion is relatively common in breast cancer.

3.3. Clinicopathologic correlation of *ING4* deletion

We then compared the clinicopathologic features of 170 breast tumors harboring *ING4* deletion to 863 tumors with no *ING4* deletion. We calculated the percentage of tumors with or without *ING4* deletion in each parameter and determined statistical significance. The results are tabulated in Table 1.

First, we found that most of the clinicopathologic features were in concordance with each other between the 2 types of tumors with no statistical differences: most tumors were ductal carcinoma (*ING4* deletion versus no deletion, 77% versus 71%), T2 (56% versus 48%), node negative (53% versus 51%), and BRE grade 2 (45% versus 42%). The median ages between breast cancer patients with and without *ING4* deletion were comparable (61 years versus 63 years). The average tumor sizes were also comparable (24 mm versus 25 mm). In addition, most tumors with *ING4* deletion were ER positive (76%), as were the tumors without *ING4* deletion (74%). The percentages of PR-positive tumors were also comparable between the 2 tumor types (47% versus 42%). Survival information was available for 956 patients of the corresponding tumor tissues evaluated by FISH (956/1033; 92.5%). We did not observe any significant differences in survival time between patients with breast cancer with or without *ING4* deletion (Table 1 and also see Fig. 3).

One distinct feature of *ING4* deleted tumors was a significant association with HER2 overexpression. Thirty-nine (23.8%) of 164 of tumors with *ING4* deletion were HER2 positive, as compared with 115 (14.1%) of 814 of tumors with no deletion (Table 1). Moreover, 25.3% (39/154) of HER2-positive tumors harbored *ING4* deletion compared with 15.1% (125/824) of HER2-negative tumors. These data showed that 1 of 4 HER2-positive tumors harbors *ING4* deletion compared with 1 of 7 HER2-negative tumors, indicating that *ING4* deletion is more prevalent in HER2-positive tumors. We compared clinicopathologic features between 39 HER2-positive *ING4*-deleted tumors with 115 HER2-positive tumors with no *ING4* deletion and observed no discernable features correlating with *ING4* deletion among HER2-positive tumors (data not shown).

3.4. *ING4* deletion is more prevalent in the HER2-positive molecular subtype of breast cancer

We examined whether *ING4* deletion was associated with any molecular subtypes of breast cancer. The molecular subtypes of breast cancer were initially defined by distinct gene expression signatures [20–22]. Subsequently, the subtypes have also been defined by the presence or absence of 3 correlative surrogate markers: ER, PR, and HER2/neu receptor (HER2) [23]: luminal A (ER+ PR+ HER2−), luminal B (ER+ PR+ HER2+), HER2 (ER− PR− HER2+), and basal-like (ER− PR− HER2−). Among our TMA tumor tissue samples, we had information regarding the status of all 3 markers in 465 tumors. Our study cohort consisted of 37.2% luminal A (173/465), 1.1% luminal B (5/465), 17.4% HER2 (81/465), and 44.3% basal-like (206/465) subtype (Table 2). These indicated a markedly lower percentage of the luminal B subtype and a higher percentage of the basal-like subtype in our study cohort, compared with other studies [24]. The reason for this skewed distribution of the subtypes in our study samples is not known.

We then determined the prevalence of *ING4* deletion in each molecular subtype of breast cancer by calculating the percentage of tumors that harbor *ING4* deletion. The results are tabulated in Table 2. In all subtypes, 15.7% contained *ING4* deletion (73/465). In the luminal A subtype, 14.5% of tumors contained *ING4* deletion (25/173), comparable with the

average *ING4* deletion rate of 15.7%. In the luminal B subtype, we could not assess the prevalence of *ING4* deletion with any statistical significance because of the small number of cohort. In the HER2 subtype, a significantly higher percentage of tumors contained *ING4* deletion (23/81; 28.4%). In contrast, only 11.7% (24/206) of the basal-like subtype tumors contained *ING4* deletion. We conclude that *ING4* deletion is more prevalent in the HER2 molecular subtype, whereas it is less prevalent in the basal-like subtype.

3.5. *ING4* deletion does not affect patient survival

We determined whether the *ING4* deletion status influenced patient survival, using subgroup analyses. The results showed no difference in overall survival rate between the patients with and without *ING4* deletion (Table 1 and Fig. 3A). *ING4* deletion did not affect the survival rate of the patients with HER2-positive tumors (Fig. 3B). We conclude that *ING4* deletion does not affect the survival rate of patients with breast cancer.

4. Discussion

We have found that *ING4* is deleted in 16.5% of breast cancer by evaluating a cohort of 1033 patient samples. This result is consistent with the previous estimate of *ING4* deletion in 10% to 20% of breast cancer [3]. We also found that *ING4* deletion was more prevalent in HER2-positive tumors and in the HER2 molecular subtype (ER− PR− HER2+) of breast cancer. Although we did not observe any effect of *ING4* deletion in patient survival either in all patients with breast cancer or in patients with HER2-positive tumors, we do not know if *ING4* deletion influences other clinical features such as response to therapy.

Breast cancer is a heterogeneous disease with the subtypes defined by distinct molecular characteristics, clinical features, and survival outcome [21,22]. HER2-positive tumors with *HER2* gene amplification or HER2/neu overexpression make up approximately 20% of all breast cancers [25]. The presence of HER2 has been correlated with high-grade tumors and associated with poor prognosis [21,22,26]. Therapy targeting HER2 such as trastuzumab has been shown effective as did monotherapy in less than 35% of patients with HER2-positive tumors [27,28]. Genetic factors that determine the responsiveness to the HER2-target therapy are unknown. It is possible that *ING4* deletion may be one of the factors that affect responses to therapy in patients with HER2-positive breast cancer. However, our clinical data regarding therapeutics were limited so that we could not address such a hypothesis.

Our results showing that *ING4* deletion is more prevalent in HER2-positive tumors suggest a suppressive role of *ING4* in the HER2-driven oncogenesis. Molecular mechanism of *ING4* antagonizing HER2 is not known. The molecular mechanism of *ING4* characterized to date involves transcription regulation via chromatin remodeling [15,29,30]. Consistently, *ING4* copurifies with chromatin remodeling complexes containing histone acetyl transferases and histone deacetylases [1,29]. In addition, *ING4* could directly bind to methylated histone H3 [30,31]. Therefore, molecular mechanism of the *ING4* tumor suppressor in HER2-positive breast cancer may involve gene regulation downstream of the HER2 receptor signal.

HER2 receptor signaling has been shown to activate the PI3K/Akt/mTOR pathways leading to the activation of the nuclear factor κB (NF-κB) transcription factor [32,33]. In glioma, ING4 was shown to modulate downstream targets genes of NF-κB [4,15]. Taken together, ING4 may suppress HER2-driven breast cancer by modulating HER2-activated NF-κB. In this case scenario, we would expect frequent deletion of *ING4* in HER2-positive tumors, which would result in constitutive activation of NF-κB. Our results showing that *ING4* deletion is more prevalent in the HER2-positive tumors are consistent with the antagonistic relationship between HER2/NF-κB and ING4.

NF-κB activation has been detected predominantly in ER-negative and HER2-positive breast cancer [34]. Biswas et al [34] also reported that NF-κB activation was rare in the basal-like molecular subtype of breast cancer (ER negative and HER2 negative). These observations are also consistent with our findings that *ING4* deletion is more prevalent in the HER2 subtype but less prevalent in the basal-like subtype of breast cancer. Thus, it appears that *ING4* deletion may correlate with NF-κB activation in breast cancer. A direct functional relationship between HER2, NF-κB, and *ING4* will require further investigation.

In conclusion, we have developed a FISH assay that can assess *ING4* deletion in breast cancer. Our results showing *ING4* deletion in 1 of 6 breast cancer reenforce a tumor-suppressive role of *ING4* in breast cancer. Furthermore, we showed that *ING4* deletion is twice more prevalent in HER2-positive tumors and the HER2 molecular subtype, suggesting that *ING4* deletion may contribute to the pathogenesis of HER2-driven breast cancer. *ING4* deletion may be used as a molecular marker to further delineate molecular subtypes in breast cancer with distinct characteristics and therapeutic implications.

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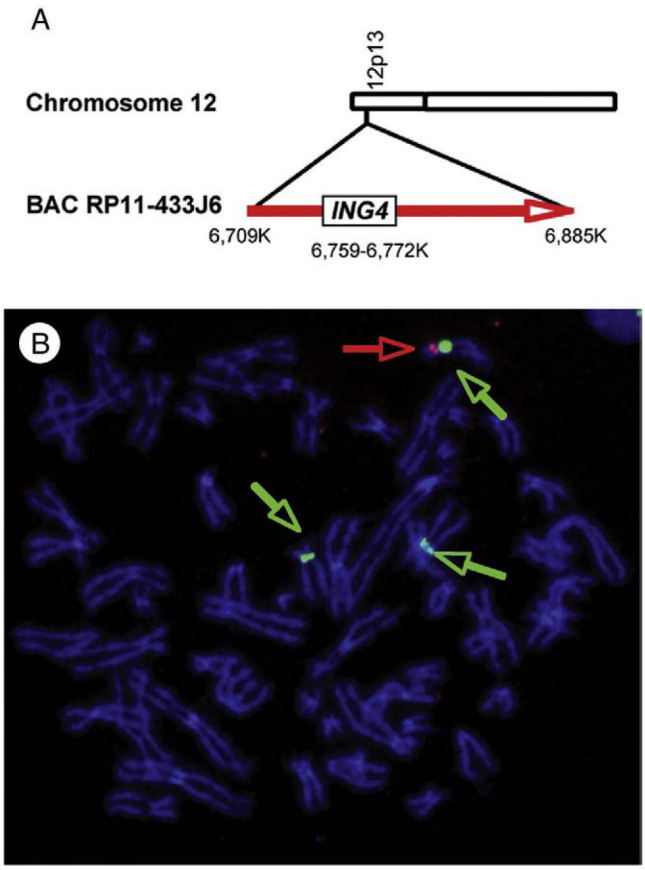

Fig. 1.

FISH detection of the *ING4* gene. A, Schematic diagram of chromosome 12, BAC RP11-433J6, and the *ING4* gene. B, T47D cells have deletions in the 2 copies of the *ING4* gene. FISH shows 3 CEP12 (green) and one copy of BAC-*ING4* (green). DNA is stained with DAPI (blue).

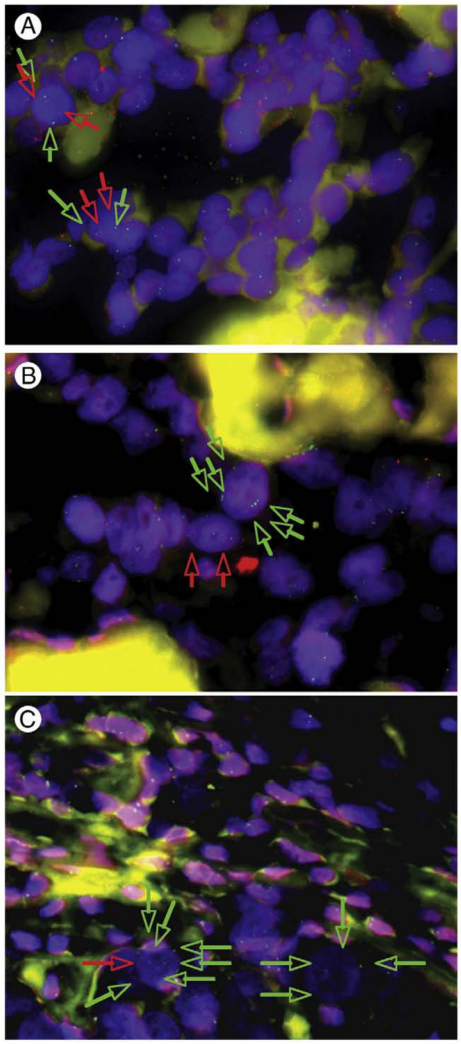

Fig. 2.

FISH on breast cancer TMA. A, Infiltrating ductal carcinoma with no *ING4* deletion shows 2 CEP12 (green; green arrows) and 2 BAC-*ING4* (BAC, red; red arrows) signals, resulting in a ratio of 1 BAC-*ING4*/CEP12. B, Ductal carcinoma with pleomorphic nuclei with *ING4* deletion shows 3 to 7 CEP12 (green; green arrows) and 0 to 3 BAC-*ING4* (red; red arrows) signals with an overall ratio of 0.38 BAC-*ING4*/CEP12. C, High-grade infiltrating ductal carcinoma with nuclear polymorphy shows an overall ratio of 0.55 BAC-*ING4*/CEP12. The nuclei contained up to 7 CEP12 (green arrows) and 0 to 2 BAC-*ING4* (red arrows). The section shows cancer cells (DAPI, blue) infiltrating the surrounding soft tissue (autofluorescent, light green).

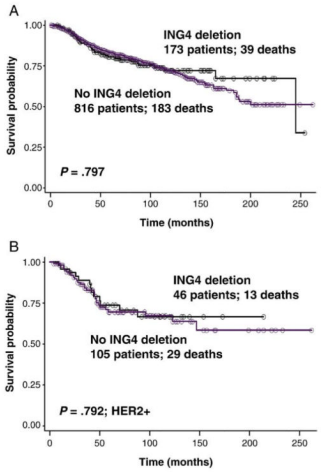

Fig. 3.

ING4 deletion does not influence a 5-year patient survival. A, Patient survival with breast cancer with (black open circle) and without (pink open circle) *ING4* deletion. B, Patient survival with HER2+ breast cancer with (black open circle) and without (pink open circle) *ING4* deletion.

Table 1

Relationship between *ING4* deletion and clinicopathologic features

Clinicopathologic feature	*ING4* (n = 1033)		*P*
	Deletion (ratio, ≤0.8) (n = 170)	No deletion (ratio, >0.8) (n = 863)	
Histologic subtype			
Ductal carcinoma	132 (77.7%)	619 (71.7%)	.113
Other	38 (22.4%)	244 (28.3%)	
Size			
pT1	51 (30.0%)	295 (34.8%)	.258
pT2	96 (56.5%)	410 (48.3%)	
pT3	8 (4.7%)	57 (6.7%)	
pT4	15 (8.8%)	87 (10.3%)	
Lymph node status			
N0	81 (53.6%)	395 (51.6%)	.652
>N0	70 (46.4%)	370 (48.4%)	
BRE			
Grade 1	26 (15.3%)	183 (21.5%)	.187
Grade 2	78 (45.9%)	358 (42.0%)	
Grade 3	66 (38.8%)	311 (36.5%)	
ER			
Positive	126 (76.8%)	627 (74.9%)	.603
Negative	38 (23.2%)	210 (25.1%)	
PR			
Positive	60 (47.2%)	273 (42.1%)	.281
Negative	67 (52.8%)	376 (57.9%)	
HER2/neu receptor			
0 + 1	125 (76.2%)	699 (85.9%)	.002
2 + 3	39 (23.8%)	115 (14.1%)	
Age at diagnosis (y), median (minimum-maximum)	61.5 (28–88)	63 (27–93)	.174
Tumor size (mm), median (minimum-maximum)	24 (4–110)	25 (0–140)	.854
Survival rate			
5 y (95% CI)	81.4% (74%–87%)	83.0% (80%-86%)	.591*
6 y (95% CI)	80.4% (72%–86%)	81.4% (78%-84%)	
10 y (95% CI)	72.6% (62%-81%)	71.8% (67%-76%)	

Abbreviation: CI, confidence interval.

* No difference in survival time throughout the duration of follow-up.

Table 2

ING4 deletion in the molecular subtypes of breast cancer

Molecular subtype	n = 465	*ING4* deletion, 73 (15.7%)	No deletion, 392 (84.3%)	*P*
Luminal A (ER+ PR+ HER2−)	173	25 (14.5%)	148 (85.5%)	<.001
Luminal B (ER+ PR+ HER2+)	5	1 (20%)	4 (80%)	NS
HER2 (ER− PR− HER2+)	81	23 (28.4%)	58 (71.6%)	.002
Basal-like (ER− PR− HER2−)	206	24 (11.7%)	182 (88.3%)	.001

Abbreviation: NS, not significant.