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SHORT COMMUNICATION

HOX gene methylation status analysis in patients with hereditary breast cancer

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Cancer development is related not only to genetic alterations but also to aberrant epigenetic changes that could lead to heritable gene patterns critical for neoplastic initiation and progression. Knowledge of epigenetic regulation in cancer cells is useful for both the understanding of carcinogenesis and for the possibility of using epigenetic drugs. *HOX* genes deregulation have a crucial role in oncogenesis process and tumor suppression. In this report, the methylation of *HOXA1*, *HOXA9*, *HOXA10*, *HOXB13*, *HNF1B*, *OTX1*, *TLX1* genes have been analyzed in patients with hereditary breast cancer. This is the first study analyzing BRCA mutational status of patients with respect to methylation of *HOX* genes. *HOXA10* has been found to be methylated in all patients analyzed but never in healthy subjects. With respect to clinical pathological information, hypermethylation of all studied genes, with the exception of OTX1, was significantly associated with absence of HER2 neu expression ($P<0.05$). Moreover, hypermethylation of *HOXB13*, *HOXA10* and *HOXA1* was associated with a high proliferation index (Mib1 $\geqslant$ 10%, $P<0.05$) and hypermethylation of *HOXB13* and *HOXA10* also with high expression of estrogen and progesterone receptors. These preliminary data suggest a possible involvement of *HOX* genes in familial breast cancer as marker helpful to identify high-risk patients.
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HOX genes have a crucial role not only in development, receptor signaling, differentiation, motility and angiogenesis, but also in apoptosis; thus, alterations in their expression could have a role in both oncogenesis and tumor suppression and, consequently, in diagnosis and therapy. In particular, *HOX* gene methylation is atypical in breast cancer tissue compared with normal epithelia[1] and is linked to malignant transformation and tumor aggressiveness.[2]

The aim of this study was to analyze the methylation status of a group of *HOX* genes in familial breast cancer to understand a possible role of their epigenetic regulation with respect to BRCA1-2 mutational status. In fact, it is already known that HOXA9 overexpression slows breast cancer progression, binding the 5′ promoter region of BRCA1 then modulating its expression.[2] For this reason, methylation of the regulatory genes *HOXA1*, *HOXA9*, *HOXA10*, *HOXB13*, *HNF1B*, *OTX1*, *TLX1* has been investigated in high-risk subjects with a familial history of hereditary breast and/or ovarian cancer, with these genes resulting highly involved in breast cancer.[3,4] This is the first study analyzing BRCA mutational status with respect to methylation of *HOX* genes in hereditary breast cancer subjects.

A total of 24 subjects (19 breast cancer affected and 5 healthy relatives from high-risk familial breast cancer) coming from the genetic counseling program of the National Cancer Research Centre of Bari, and a pool of 9 healthy unrelated controls without breast cancer were enrolled. Among the group of 24, subjects #10, #11, #12, #13 and #20 were healthy, BRCA mutated and with a strong history of breast cancer, whereas patients #3, #8 and #23 also had ovarian cancer.

All subjects were tested by the Methyl-Profiler DNA methylation PCR System (Qiagen GmbH, Hilden, Germany) for the methylation status of *HOX* genes mentioned above. The method was based on the detection of remaining input DNA after cleavage with a methylation-sensitive enzyme and/or a methylation-dependent restriction enzyme and quantified by real-time PCR. All subjects were analyzed for BRCA1 and BRCA2 mutational status[5] and all molecular and clinical pathological information is reported in Table 1.

Healthy controls were unmethylated in all the investigated genes.

The tests highlighted a hypermethylation of the *HOXA10* gene in all high-risk subjects. Silencing of *TLX1*, *OTX1*, *HOXA9*, *HOXA1* and *HNF1B* genes by methylation has been found to be significantly associated with BRCA mutational status considering both patients and healthy subjects with a high risk of familial breast cancer history ($P=0.01$) (Table 2). Hypermethylation of all studied genes, with the exception of *OTX1*, was significantly associated with absence of HER2 neu expression ($P<0.05$). Moreover, hypermethylation of HOXB13, HOXA10 and HOXA1 was associated with a high proliferation index (Mib1 $\geqslant$ 10%, $P<0.05$) and hypermethylation of HOXB13 and HOXA10 also with high expression of estrogen and progesterone receptors.

The goal of this study was to analyze the *HOX* gene methylation in heredo-familial breast cancer, particularly focusing on the possibility

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Table 1 Clinicopathological features of the subjects belonging to high-risk families, affected and not affected by breast cancer

ID	Gene mutated	Sex	Disease	ER	PgR	Mib1	Her2	Grading	Histology
1	*BRCA1*	F	BC	0	0	50	0	3	IDC
2	*BRCA1*	F	Bil-BC	70	0	25	0	3	IDC
3	*BRCA2*	F	BC + OC	60	80	30	0	3	IDC
4	*BRCAX*	F	BC	15	20	40	+	2	IDC
5	*BRCAX*	F	BC	0	0	30	+	2	IDC
6	*BRCA2*	F	Bil-BC	40	25	0	0	2	IDC
7	*BRCAX*	F	BC	0	10	30	+	2	IDC
8	*BRCA1*	F	BC + OC	30	80	45	0	3	IDC
9	*BRCA2*	F	BC	60	15	10	0	3	IDC
10	*BRCA1*	M	H	—	—	—	—	—	—
11	*BRCA1*	F	H	—	—	—	—	—	—
12	*BRCA2*	F	H	—	—	—	—	—	—
13	*BRCA2*	M	H	—	—	—	—	—	—
14	*BRCAX*	F	BC	0	0	NA	+	3	IDC
15	*BRCAX*	F	BC	0	0	20	+	2	IDC
16	*BRCA2*	F	BC	20	20	20	0	3	IDC
17	*BRCA1*	F	BC	0	0	38	0	3	IDC
18	*BRCAX*	F	BC	80	10	20	0	2	IDC
19	*BRCA2*	F	BC	0	0	0	0	2	IDC
20	*BRCA1*	F	H	—	—	—	—	—	—
21	*BRCAX*	F	BC	0	0	80	0	3	IDC
22	*BRCAX*	F	BC	80	70	10	0	3	IDC
23	*BRCA1*	F	BC + OC	0	0	NA	0	2	IDC
24	*BRCA2*	F	BC	45	35	18	0	3	IDC
Pool of 9 healthy people	—								

Abbreviations: BC, breast cancer; Bil-BC, bilateral breast cancer ; H, healthy with high-risk familial breast cancer; IDC, intraductal carcinoma; NA, not analyzed; OC, ovarian cancer.

Table 2 Methylation status of the high-risk subjects affected and not affected by breast cancer ($n = 24$)

Gene	Methylation status	Number	BRCA1-2 mutated	BRCAX	P
HoxA1	Yes	6	4	2	0.01
	No	18	12	6	
HoxA9	Yes	9	6	3	0.01
	No	15	10	5	
HoxA10	Yes	24	16	8	n.s.
	No	0	—	—	
HoxB13	Yes	21	14	7	n.s.
	No	3	2	1	
HNF1B	Yes	7	6	1	0.01
	No	17	10	7	
OTX1	Yes	8	6	2	0.01
	No	16	10	6	
TLX1	Yes	6	4	2	0.01
	No	18	12	6	

Abbreviation: n.s., not significant.

to use them as susceptibility markers in subjects with a high risk of hereditary breast cancer but without BRCA1 and BRCA2 mutations. This is the first experimental approach analyzing *HOX* gene methylation in patients with a strong familial history of breast/ovarian cancer compared with BRCA1/2 mutational status.

All previous published studies show that *HOX* genes are misregulated in cancer, but the role of their hypomethylation or hypermethylation is not clear.[6]

In this series, HOXA10 resulted in hypermethylation in all high-risk subjects, affected and not, with no differences between mutated and non-mutated cases, whereas all healthy controls were unmethylated, thus assuming a possible role of this gene as a susceptibility marker in BRCAX (without mutations in *BRCA1* and *BRCA2* genes) patients. HOXA10 was reported to regulate p53 expression and invasion in breast tumor cells[7] and to be lower expressed in breast tumors compared with normal tissue;[8] however, no data were available on HOXA10 expression and familiarity.

Previous studies showed an association between *HOX* gene methylation and immunophenotypes showing a close association between HOXA1 and HOXA10 methylation and infiltrating ductal carcinoma (IDC). In this series, all cases analyzed were IDC, confirming the behavior of HOXA10. Regarding HOXA1, Park *et al.*[9] showed how HOXA1 hypomethylation resulted significantly present in basal-like tumours apparently in contrast with the association between BRCA mutations and hypermethylation of TLX1, OTX1, HOXA9, HOXA1 and HNF1B described here. Currently, only 37.5% of BRCA1 mutated cases are triple-negative breast cancer and we do not have any information on other markers characterizing basal-like tumors (Table 1).

The significant silencing of HOXB13 and HOXA10 in ER- and PgR-positive tumors confirmed promoter hypermethylation of HOXB13 more frequently observed in ERa-positive patients[10] and regulation of HOXA10 by the complex E_2-ER and its modulation by ERE elements.[11]

No previous data have been published about the correlation between methylation status of *HOX* genes and proliferative activity (Mib1 expression) or HER2 neu expression here described.

OTX1 has been shown to be regulated by p53 only in cancer stem cells but not in physiological conditions, while the interaction of these two genes is involved in the differentiation of cancer stem cells as a tumor suppression mechanism.[12]

Three patients also presented ovarian cancer but, being only a few cases, no peculiar characteristics on methylation pattern emerged. Kelly *et al.*[13] reviewed different studies about the *HOX* gene behavior in ovarian cancer. Furthermore, HOXB13 has been demonstrated to induce ovarian cancer progression but also to be a functional regulator of antihormonal resistance in human ovarian cancer.[14]

In the present report, the authors hypothesized that the analysis of methylation status of *HOX* genes in familial breast and/or ovarian cancer could be a milestone to individuate novel markers in this series of selected patients. The reported preliminary data, in fact, highlighted the involvement of *HOXA10* gene inactivation as a marker of familial breast cancer. Furthermore, association between specific *HOX* gene methylation and BRCA mutations and histopathology and clinical aggressiveness parameters suggest the possibility of identifying patients at higher risk.

Many questions remain still unclear and other studies will be necessary to confirm our data and clearly define the role of *HOX* gene inactivation in the hereditary breast cancer phenotype.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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