

VIEWPOINT

BRCA1: linking HOX to breast cancer suppression

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Abstract

Homeobox (*HOX*) genes play key roles in embryogenesis and tissue differentiation. Recently, a number of groups have reported altered *HOX* gene expression in breast cancer. However, the mechanism of *HOX* gene regulation and the search for direct targets of its transcriptional regulatory function have been minimally fruitful. Recently, Gilbert and colleagues reported that HOXA9 restrains breast cancer progression by upregulation of *BRCA1*, a tumor suppressor. This finding raises our hope that more, rather elusive targets of *HOX* genes important in tumor progression or suppression will be found in the future.

Background

Homeobox (*HOX*) genes, a family of transcription factors, function as key determinants of antero-posterior patterning of animal embryos and the development of several organs [1]. Formed by gene duplication, 39 members of the *HOX* gene family are arranged in four *HOX* clusters, A, B, C, and D on human chromosomes 7, 17, 12, and 2, respectively. Altered *HOX* gene expression has been implicated in differentiation, invasion, epithelial-mesenchymal transition, apoptosis, and receptor signaling in a variety of cancer types [2-8]. In breast cancer, some *HOX* genes are overexpressed whereas others are underexpressed, and mutations are rarely observed [9]. However, the molecular mechanism of *HOX* gene action has remained elusive. Ten years after a study by Raman and colleagues [4] demonstrated that HOXA5 can control *p53* transcription and that loss of HOXA5 correlated with loss of *p53* expression in breast cancer, a study by Gilbert and colleagues [10] has pinpointed another gene critical for breast cell function, *BRCA1*, as the target of a *HOX* gene. In the latter study, the authors

present strong evidence that HOXA9 inhibits breast cancer progression by modulating expression of *BRCA1*.

The paper

HOXA9 as a breast cancer-related gene piqued the interest of this group when an Affymetrix microarray analysis (Affymetrix, Santa Clara, CA, USA) of five paired sets of microdissected breast tumors and their adjacent normal tissue identified HOXA9 as 1 of 115 transcripts that showed tumor-specific downregulation. Examination of publicly available databases showed that HOXA9 is significantly downregulated in breast cancer and correlated with disease aggressiveness. The authors then went on to investigate the effects of HOXA9 re-expression on tumor cell proliferation, survival, and differentiation. Interestingly, overexpression of HOXA9 in estrogen receptor-negative cell lines inhibited breast cancer cells through decreases of cell growth, survival, and invasiveness and changes in morphogenesis. In addition, to find targets of HOXA9, the authors performed global transcriptional profiling of HOXA9-overexpressing MDA-MD-231 cells. They found that the expression of *BRCA1*, the breast cancer susceptibility gene and a well-known tumor suppressor gene, was higher in HOXA9-overexpressing cells compared with vector control cells. By performing chromatin immunoprecipitation analysis, they found that HOXA9 directly binds to the 5' promoter region of *BRCA1*. If HOXA9 is a regulator of tumor suppressor genes, they reasoned, loss of HOXA9 in nonmalignant MCF-10A mammary epithelial cells should lead to malignant transformation. To test this concept, the authors depleted HOXA9 in MCF10A cells with short hairpin RNAs (shRNAs). This led to increases of cell growth, invasiveness, and survival phenotype. Similar changes were seen upon knockdown of *BRCA1*. Co-expression of HOXA9 with mutants of *BRCA1* reduced the ability of HOXA9 to inhibit tumorigenesis of breast cancer cells. In addition, by immunohistochemistry studies, the authors provide clinical evidence that there is a strong positive association between expression of HOXA9 and that of *BRCA1*. In summary, Gilbert and colleagues [10] demonstrated that HOXA9 inhibits cell proliferation and survival of human breast cancer cells, possibly by upregulation of *BRCA1*. These findings provide an explanation for the

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concomitant loss of HOXA9 and BRCA1 expression in breast cancer.

Viewpoint

Previous studies have shown that *HOXA9* is highly methylated and downregulated in several types of cancer, including breast cancer, but the consequences of this loss were not explored [11]. The article by Gilbert and colleagues [10] clarifies, for the first time, the clinical correlation and relevance of loss of HOXA9 and breast tumorigenesis. Second, the demonstration that shRNA-mediated knockdown of HOXA9 promotes tumorigenesis in nonmalignant mammary epithelial cells supports the inference that, in normal cells, HOXA9 inhibits cell proliferation and survival of human breast cancer cells, possibly by upregulation of BRCA1. However, many questions remain. Can the loss of the HOXA9 gene alone disrupt homeostasis in breast cells and initiate/promote tumorigenesis? Do our experiments in tissue culture faithfully reflect *in vivo* events? In 1999, Chen and Capecchi [12] reported mild to no phenotypic changes in mammary glands of *HOXA9/HOXB9/HOXD9* knockout mice; these triple-knockout female mice showed hypoplasia of the mammary glands but only upon induction of pregnancy and lactation; however, they did not develop tumors. In the article of Gilbert and colleagues [10], the arrayed tumor samples were from five individuals ranging in age from 44 to 54 years and were presumably parous. Did parity play a role in heightening the effects of the loss of HOXA9 in their breast cells? Are HOXA9 and BRCA1 also downregulated in nulliparous patients with breast cancer? Why do the other paralogs – HOXB9, C9, or D9 – not substitute for the function of the lost HOXA9? Perhaps HOXA9 is the only paralog specifically expressed in breast epithelial cells. Interestingly, as mentioned by the authors, HOXA9 is overexpressed and activated in acute myeloid leukemia and endothelial cells. But these cells do not show concomitantly high BRCA1 expression [13,14], supporting the dogma that HOX target genes are cell- and tissue-specific. In conclusion, the findings in this paper are very informative, establishing the ability of HOXA9 to exert its vigilante function through regulating the expression of BRCA1 in breast cells. The paper raises

as many questions as it answers – a hallmark of an interesting paper.

Abbreviations

HOX, homeobox; shRNA, short hairpin RNA.

Competing interests

The authors declare that they have no competing interests.

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