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Twist overexpression promotes chromosomal instability in the breast cancer cell line MCF-7

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Erroneous expression of genetic information is a characteristic of a transformed phenotype in cancer biogenesis [1]. The degree of chromosomal instability in a cell can determine its fate toward proliferation or cell death. Chromosomal instability manifests as aneuploidy (loss or gain of chromosomes) or as a rearrangement of chromosomal structure. For example, in human Burkitt's lymphoma, the *C-MYC* oncogene is translocated downstream of the enhancer of the immunoglobulin heavy chain gene, resulting in overexpression of *C-MYC*, which increases both the rate of cell division and chromosomal instability [2]. The translocation resulting in the production of the chimeric BCR-ABL fusion protein has been demonstrated to transform hematopoietic cells, resulting in chronic myelogenous leukemia in humans [3]. Thus, chromosomal aberrations can result in overexpression of oncogenes or suppression of tumor suppressor genes, which in turn promote oncogenic transformation.

Fluorescence cytogenetic methods have been well established as a way of studying chromosomal instability, and include techniques such as spectral karyotyping (SKY) and comparative genomic hybridization (CGH). SKY permits the simultaneous visualization of each mammalian chromosome in a different fluorescent color, facilitating the identification of both structural and numerical chromosomal aberrations [4–6]. CGH reveals the different hybridization patterns of labeled tumor versus control (reference) DNA and generates a map of DNA copy number changes in tumor genomes [7]. CGH has been consistently used to characterize chromosomal aberrations in solid tumors and hematologic malignancies in patients [8,9]. It is very challenging, however, to develop a tumor model system that will allow for the correlation between the function(s)/expression of a particular protein in vivo and its ability to induce chromosomal instability. This is primarily due to the fact that tumors are heterogeneous and the phenotype observed results from the interplay of a number of proteins acting in concert.

The basic helix-loop-helix transcription factor Twist is a major regulator of mesenchymal phenotypes. It has been shown that loss of appropriate levels of expression or mutations of *Twist* result in developmental defects [10]. More recently, it has been demonstrated that Twist overexpression correlates with high-grade breast carcinomas [11]. To further characterize the functions of Twist in cancer biogenesis, we have generated a human breast cancer cell line that stably over-expresses human Twist (MCF-7/Twist). The overexpression of Twist causes an epithelial to mesenchymal-like transition (EMLT) leading to increased invasiveness and motility of this cell line [11,12]. This phenotypic transformation caused by Twist overexpression is the result of altered gene expression levels and profiles within the cells.

To characterize the cytogenetic changes induced by Twist overexpression, we analyzed the MCF-7/Twist cell line by SKY. As seen in Fig. 1, we found a significant number of chromosomal abnormalities and structural aberrations in the MCF-7/Twist cell line compared to the MCF-7 vector control cells. Aneuploidy was observed in all chromosomes except 2,3,12,18, and 21. In addition, structural aberrations and translocations were found in all but

two chromosomes (4 and 18). This would indicate that Twist, in some capacity, promotes chromosomal instability in the MCF-7 cell line. This finding validated our earlier observations of human breast tumor samples, which exhibited increased chromosomal abnormalities in Twist-expressing tumors compared to nonexpressers [11]. Of the 144 breast tumor samples analyzed by CGH, we found that there were, on average, 14.1 cytogenetic alterations in the Twist-expressing tumors compared to 7.1 alterations in the Twist nonexpressing tumors ($P < 0.05$). We also found chromosomes 1, 7, 15, and 17 to be amplified only in the Twist-expressing tumors. Similar results were also observed in MCF-7/Twist cell line (Fig. 1). The amplification of these chromosomes has been reported in breast cancer and they harbor a variety of oncogenes dysregulated in the process [13,14]. Both these findings (in MCF-7/Twist cells and in patient samples) clearly demonstrate that Twist overexpression plays a role in destabilizing the genome, thus promoting chromosomal instability. The fact that the MCF-7/Twist cell line has more chromosomal instability than the tumors is possibly the result of a selection bias during the generation of a stable clone rather than a tumor progression event. To our knowledge, this is the first such report that shows a direct correlation between overexpression of Twist and increased chromosomal instability in both tissue culture cells and patient breast tumors. The data we have obtained using SKY in a transgenic cell line clearly indicates the importance of associating gene functions observed in patient tumors to that in tissue culture cells. This validation is crucial to the understanding of the functions of a gene (in this instance, Twist) to promote chromosomal instability and augment the breast tumorigenesis process.

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MCF-7 vector control

MCF-7/Twist

Fig 1. Spectral karyotyping of MCF-7 vector control and MCF-7/Twist cells

The dashed squares (white) represents gain of chromosomes (1, 7, 15, and 17) in MCF-7/Twist cells, which mirrors that observed in Twist over-expressing breast tumor patient samples but not in MCF-7 vector control cells.