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14. ABSTRACT
The development of breast cancer is associated with gene amplification and overexpression that are believed to have a causative role in oncogenesis. An important challenge in breast cancer research is to identify and characterize these genetic changes. Focal amplifications involving chromosome 8p11-p12 occur in approximately 15~20% of primary, uncultured human breast cancers. Recently, we have undertaken a detailed genomic and expression analysis of the 8p11-p12 amplicon in breast cancer cell lines and identified several novel candidate genes including TC-1 and FLJ14299. We observed that TC-1 is located at the common core-amplified domain of the 8p11-12 region and overexpressed in the subset of breast cancer cells. Furthermore, we have found that TC-1 has properties of an oncogene: TC-1 expression in normal mammary epithelial cell line MCF10A increases growth rate and allows growth in soft agar. Notably, suppression of TC-1 expression by siRNA inhibited cell proliferation in TC-1 over expressing breast cancer cell lines. Our recent date also suggested that TC-1 over-expression is associated with the enhanced expression of a subset of beta-catenin target genes in breast cancer.

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Table of Contents

Zeng-Quan Yang, Ph.D.
Annual Report for Award DAMD 17-03-1-0459
June 21, 2006

Introduction:

The development of breast cancer is associated with gene amplification and overexpression that are believed to have a causative role in oncogenesis(1-4). An important challenge in breast cancer research is to identify and characterize these genetic alternations. Global genomic and transcriptomic analysis have led to important insights in our understanding of the complexity and heterogeneity of this disease, and providing new avenues for the discovery of genetic alternation in human breast cancer (HBC). Recently we have performed the cytogenetic and molecular genetic profile of the SUM panel breast cancer cell lines. Of specific relevance to this application, we have found that three cell lines have overlapping amplicons in the short arm of chromosome 8. From this amplicon, we identified several novel candidate genes including TC-1 (C8ORF4), FLJ14299 and others(5). TC-1 is a novel gene highly expressed in thyroid cancer and some fraction of breast cancers. FLJ14299 contains a C2H2-like motif, which is also present in several tumor-related genes. Aberrant expression of TC-1 and FLJ14299 could be related to development and progression in breast cancer. The specific aims as outlined in this proposal will help us to better understand the biological function and genetic pathway of new target genes of this amplicon and identify better prognostic and predictive markers for an important subset of breast cancer.

Body:

Task 1. To test the mechanistic significance of two novel genes, *FLJ14299* and *TC-1*, found to be amplified and over expressed in the SUM-44, SUM-52 and SUM-225 breast cancer cell lines (Months 1-20)

In our previous annual report, we demonstrated that TC-1 has oncogenic properties. Overexpression of TC-1 in a spontaneously immortalized normal mammary epithelial cell line, MCF10A, enhanced cell proliferation in tissue culture. More important, TC-1 overexpression significantly promoted colony formation in soft agar assay relative to control vector alone. These results support the notion that TC-1 can facilitate cell growth and anchorage-independent growth associated with transformed phenotypes. Overexpression of FLJ14299 in MCF10A cells did not enhance cell proliferation and anchorage-independent growth. In addition, suppression of TC-1 expression by siRNA inhibited cell proliferation in TC-1 over expressing breast cancer cell lines.

In order to investigate the individual and cooperating oncogenic properties of newly identified genes in 8p11-12 amplicon, we established a cDNA expression library containing 8 candidate genes including TC-1 and FLJ14299 using a lentiviral expression system in this year. We transduced cells with several different combinations of genes from the lentiviral library into MCF10A cells and selected recipient cells in serum-free

medium lacking EGF or IGF. RT-PCR with genes and vector-specific primers were used to detect transduced genes in cell clones that acquired EGF independence. Our primary data revealed that a combination of TC-1 and one of other two genes was sufficient to induce EGF independent growth (Manuscript in preparation). Clones containing only one of the three aforementioned genes were unable to survive in EGF-free medium. This result suggested that cooperative activity of oncogenes including TC-1 within amplicons is crucial for inducing the specific transforming phenotypes in breast cancer.

Task 2. To detect potential downstream target genes of *TC-1*and *FLJ14299* overexpression using human cancer array and to investigate their particular tumor-related biological phenomena (Months 8-36)

TC-1 is a novel gene that was originally cloned from suppression subtractive hybridization between papillary thyroid carcinoma and its surrounding normal thyroid tissue(6). Recently, the structural characterization of the TC-1 protein revealed it is a natively disordered protein(7). Disordered proteins have been suggested to play roles in cell-cycle control, signal transduction, transcriptional and translational regulation. Recently, Jung et al. have reported that TC-1 is a positive regulator of the beta-catenin pathway in gastric cancer. TC-1 interacts with Chibby (Cby), which negatively regulates beta-catenin–mediated transcription. Thus, TC-1 enhances beta-catenin signaling by relieving the suppression induced by Cby. The expression of TC-1 was also analyzed using tissue microarray in correlation with beta-catenin target genes in 299 gastric cancers. To test whether TC-1 induces beta-canenin target genes in human breast cancer, we performed Affymetrix U133-Plus-2 array in our MCF10A-TC-1 and SUM-52-pSR-TC-1 RNAi cells. We found that TC-1 induced a subset of beta-catenin target genes including CCND1, CLDN1, EDN1, MITF and MMP7 in breast cancer. These results were confirmed by quantitative RT-PCR. Thus, TC-1 has transforming properties and its over-expression is associated with the enhanced expression of a subset of beta-catenin target genes in breast cancer.

Task 3. To determine the amplification and overexpression pattern of 8p11-12 genes in primary breast cancer and to determine their associations with tumor phenotype and prognosis (Months 6-36)

To determine the amplification pattern of 8p11-12 genes in primary breast cancer, quantitative PCR analysis was carried out using genomic DNA obtained from 90 breast cancer specimens. The PCR experiments were performed using primers specific for *FLJ14299, LSM1, FGFR1* and *TC-1,* as these genes span the 8p11-12 amplicon detected in the cell lines(5). Of the 90 breast cancers examined, 25 showed evidence of high level amplification (greater than 4-fold) in at least part of the 8p11-12 region. Interestingly, *TC-1* and *FLJ14299* were most commonly amplified, while *FGFR1* was only found to be greater than 4-fold amplified in 4 of 90 primary breast cancers. These results suggest that genes such as TC-1 and FLJ14299 flanking the *FGFR1* locus may be of greater significance in breast cancers.

To elucidate the significance of TC-1 expression in primary HBC, we performed quantitative RT-PCR experiments using RNA isolated from the primary HBC specimens. In 29 samples analyzed, 7 samples had TC-1 gene amplification by array CGH, and/or quantitative genomic PCR. In these 29 samples,thirteen samples (45%) exhibited an up-regulated of TC-1 mRNA level (>4 fold) when compared with the normal breast tissue. We did not find that the mRNA level correlated positively with the DNA amplification level in both breast cancer cell lines and primary HBC specimens. There were no clear clinical features that were associated with TC-1 mRNA expression level.

Training accomplishments:

With the help of this training award, my ability in critical thinking and problem solving in research work, the skills needed to formulate and carry out research, and to report findings in peer-reviewed journals have been dramatically improved. From October 1, 2004, I became an Research Assistant Professor in the Department of Pathology, Wayne State University School of medicine, and a member of the Breast cancer research program of the Karmanos Cancer Center.

Key Research Accomplishments:

1, Novel gene TC-1 of 8p11-12 amplicon has oncogenic properties in breast cancer.

2, TC-1 induced a subset of beta-catenin target genes including CCND1, CLDN1, EDN1, MITF and MMP7 in breast cancer.

3, TC-1 mRNA levels were frequently up-regulated in breast tumors.

Reportable Outcomes:

Manuscripts:

Yang Z-Q , Streicher K, Ray M, Abrams J and Ethier S. Multiple interacting oncogenes on the 8p11-p12 amplicon in human breast cancer. Cancer Cell (Submitted)

Yang Z-Q , Ray M and Ethier S. TC-1: a novel regulator of FGFR and beta-catenin pathway in breast cancer (Preparation)

Abstracts:

1. Yang Z-Q , Streicher K, Dombkowski and Ethier S. Integrative genomic analysis identify novel oncogenes in breast cancer. 2006 American Association for Cancer Research Annual Meeting in Washington, DC. April 1-5, 2006.
2. Yang Z-Q and Ethier S. Identification and characterization of novel oncogenes in breast cancer. Post Genomics Summit 2006 in Beijing China, May 17-19, 2006

Conclusions:

We have made significant progress in the past year in characterizing two novel genes, TC-1 and FLJ14299, within 8p11-12 amplicon of breast cancer. we found that TC-1 has transforming properties and its over-expression is associated with the enhanced expression of a subset of beta-catenin target genes in breast cancer. Furthermore, we found that TC-1 expression is mechanistically linked to the FGFR2 pathway in a fraction of breast cancers. Thus TC-1, a novel regulator of FGFR2 and beta-catenin pathways, may be implicated in the progression and development of breast cancer by the up-regulation of β-catenin target genes.

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