SHORT COMMUNICATION

APC truncation and increased β-catenin levels in a human breast cancer cell line

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Mutations in the Adenomatous Polyposis Coli (APC) tumor suppressor gene or the β-catenin gene are present in most colon cancers and less frequently in other tumor types. In this study, we screened 24 human breast cancer cell lines and three immortalized human breast epithelial cell lines for alterations in β- and γ-catenin and APC by western blotting, protein truncation assay and DNA sequence analysis. In one cell line (DU 4475), an APC mutation was identified (E1577stop) that resulted in expression of truncated APC. This mutation was associated with elevated cytosolic β-catenin levels, probably due to loss of APC function, as in colon cancers. No mutations were found in exon 3 of the β- or γ-catenin genes. We conclude that APC mutations and β-catenin upregulation may occur with low frequency in human breast cancer cells.

β-Catenin, a key cytosolic component of the Wnt signal transduction pathway, is implicated in embryonic development and carcinogenesis. Normally, cytosolic β-catenin is maintained at low levels by the APC gene product, which promotes β-catenin degradation, and by cadherins, which integrate β-catenin into adherens junctions (1). Wnt signal activation results in stabilization of β-catenin (2–4) and subsequent translocation of β-catenin to the nucleus, where it binds to TCF-class transcriptional factors and induces expression of TCF-responsive genes (5, 6). γ-Catenin has structural and functional similarities to β-catenin (1) but its role in Wnt signaling has not been elucidated. Several reports have documented that deregulation of the Wnt pathway is associated with human tumorigenesis. Truncations of the APC protein are linked to the familial adenomatous polyposis (FAP) coli syndrome and are found in the majority of sporadic colon carcinomas (7). Mutations in either APC or β-catenin, resulting in deregulation of β-catenin turnover, increase β-catenin/Tcf signaling in colon cancer (8) and melanoma cell lines (9).

The APC encoding locus on chromosome 5q21 shows loss of heterozygosity (LOH) in ~25% of breast cancers (10). Frequent alterations in expression of E-cadherin and α- and β-catenins have been reported in a survey of 18 human breast cancer cell lines (11). High Wnt gene expression (Wnt-2, Wnt-4, Wnt-7b) has been seen in human proliferative breast lesions (12). Overexpression of Wnt-1 and Wnt-3 in the murine mammary gland induces mammary hyperplasia and increases the incidence of mammary gland tumors (1). In addition, APCmm mice, which represent a model for the human FAP syndrome, have an increased incidence not only for intestinal neoplasias but also for mammary tumors (13). Despite the link between Wnt signal activation and mammary gland tumors in the mouse, comparable signal activation due to mutations in genes encoding Wnt signaling components has not been documented in human breast cancer. In the present study, we screened a panel of 27 cell lines, either derived from human breast carcinomas or established by immortalization of normal breast epithelial cells, for alterations in the β-catenin, γ-catenin and APC genes and for mutations in the APC, β- and γ-catenin genes.

Cell lines BT 20, BT 474, BT 483, BT 549, CAMA 1, DU 4475, HBL 100, HS 578 T, MCF7, MCF10A, MDA-MB 157, MDA-MB 175 VII, MDA-MB 231, MDA-MB 330, MDA-MB 361, MDA-MB 415, MDA-MB 435S, MDA-MB 436, MDA-MB 453, MDA-MB 468, SKBR 3, T 47 D, UACC 812, UACC 893, ZR 75-1 and ZR 75-30 were purchased from the American Type Culture Collection (ATCC; Rockville, MD). MCF10A is a spontaneously immortalized line from normal human breast epithelial cells, and HBL 100 is an SV40 immortalized human breast epithelial cell line. HB2, another SV40 immortalized human breast epithelial cell line, was obtained from Dr Joyce Taylor-Papadimitriou (London, UK). Cells were grown under recommended conditions and harvested at 90–100% confluency. For total cell protein extraction, cells were lysed in TENT buffer (50 mM Tris pH 8.0, 1 mM EDTA, 150 mM NaCl, 1% Triton X-100), centrifuged, the supernatant at low levels by the APC gene product, which promotes β-catenin degradation, and by cadherins, which integrate β-catenin into adherens junctions (1). Wnt signal activation results in stabilization of β-catenin (2–4) and subsequent translocation of β-catenin to the nucleus, where it binds to TCF-class transcriptional factors and induces expression of TCF-responsive genes (5, 6). γ-Catenin has structural and functional similarities to β-catenin (1) but its role in Wnt signaling has not been elucidated. Several reports have documented that deregulation of the Wnt pathway is associated with human tumorigenesis. Truncations of the APC protein are linked to the familial adenomatous polyposis (FAP) coli syndrome and are found in the majority of sporadic colon carcinomas (7). Mutations in either APC or β-catenin, resulting in deregulation of β-catenin turnover, increase β-catenin/Tcf signaling in colon cancer (8) and melanoma cell lines (9).

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To analyze the entire β-catenin gene from DU4475, cDNA was synthesized in 12 separate RT–PCR reactions (primer sequences available upon request) using total RNA as template. cDNA was then PCR-amplified, submitted to automated sequencing and compared to the published β-catenin sequence.
Fig. 2. β- and γ-catenin levels in the cytosolic fraction of selected breast cancer cell lines, analyzed by western blot. HB2 serves as control. Note increased content of cytosolic β-catenin in DU 4475.

Fig. 3. (A) Protein truncation assay of segment B (codons 1099 to 1693) of the APC gene. The control was generated with a human genomic DNA sample known to have the wild type APC gene. A truncated protein is seen as an aberrant band in DU 4475. Representative for all other breast cancer cell lines, reaction products were submitted to electrophoresis on a precast 6% polyacrylamide–7 M urea gels and visualized by autoradiography (BioMax; Kodak, Rochester, NY). All experiments were performed at least twice.

Total cell lysate western blot analysis (Figure 1) showed strong expression of β-catenin in BT 20, BT 474, BT 483, BT 83, DU 4475, HB2, HBL 100, HS 578 T, MCF7, MCF10A, MDA-MB 157, MDA-MB 175 VII, MDA-MB 231, MDA-MB 361, MDA-MB 415, MDA-MB 453, MDA-MB 468, SKBR3, T 47 D, UACC 812, UACC 893, ZR 75-1 and C57-Wnt-1 (positive control); weak expression was seen in CAMA 1, MDA-MB 435 S, MDA-MB 436, MDA-MB 453 and UACC 812; β-catenin was detected at very low levels in MDA-MB 330, SKBR 3 and ZR75-30. In DU 4475, β-catenin expression was extremely strong, exceeding the positive control (Figure 1A). In MDA-MB 468, a double band was seen with a second signal at ~80 kDa (Figure 1A). γ-Catenin expression was strong in BT 20, BT 474, BT 483, BT 549, CAMA 1, DU 4475, HB2, HBL 100, MCF7, MCF10A, MDA-MB 157, MDA-MB 175 VII, MDA-MB 231, MDA-MB 361, MDA-MB 415, MDA-MB 453, MDA-MB 468, SKBR3, T 47 D, UACC 812, UACC 893 and ZR 75-1; weak in HS 578 T. MDA-MB 231, MDA-MB 435 S, MDA-MB 436, MDA-MB 453, ZR 75-30 and C57-Wnt-1; and not detectable in MDA-MB 330 (Figure 1B). All detectable β- and γ-catenin signals migrated at the expected position for an apparent molecular weight of 92 and 82 kDa, respectively.
On the basis of these results, the following cell lines were selected for analysis of cytosolic (Figure 2) and membranous protein fractions (data not shown): CAMA 1 (weak expression of β-catenin), DU 4475 (high expression of β-catenin), HS 578 T (low levels of γ-catenin), MDA-MB 468 (β-catenin double band), SKBR 3 (low levels of β-catenin) and HB2 (control). As expected, in most lines β- and γ-catenin levels were very low in the cytoplasmic fraction (Figure 2), whereas both proteins were prominent in the membranous fraction (data not shown). Of the cell lines analyzed, β-catenin was significantly elevated in the cytosolic fraction of DU 4475 (Figure 2). γ-Catenin levels were slightly stronger in the cytosolic fraction of DU 4475, as compared with the other cell lines analyzed, but not to the extent exhibited for β-catenin. Low levels of β-catenin in CAMA 1 (Figure 1) were confirmed in the fractionated analysis (Figure 2).

We analyzed the serine/threonine region encoded by exon 3 of the β- and γ-catenin genes from all cell lines and the entire β-catenin gene from DU 4475. However, no mutations were identified in our study.

The protein truncation assay was performed on two APC segments encompassing codons 686 through 1693 for all cell lines listed except HB2, MCF10A, MDA-MB 330 and MDA-MB 435S. Of all protein products, one showed an aberrant size: The segment B product from DU 4475 had an apparent molecular weight of 70 kDa instead of the expected 100 kDa (Figure 3A). No wild-type allele, or full length protein product, was detected in this cell line (Figure 3A). Sequencing of the respective APC gene segment revealed a point mutation at position 4747 of the coding sequence (G→T), converting codon 1577 into a stop codon (E1577stop) (Figure 3B).

We analyzed 24 human breast carcinoma cell lines and three immortalized human breast epithelial cell lines for alterations in β-γ-catenin and APC. Mutations in the APC or β-catenin genes that result in accumulation of β-catenin have been reported in several carcinoma cell lines (8,9,15,16). We hypothesized that similar aberrancies may play a role in human mammary tumorigenesis. Our analysis focused on cancer cell lines to correlate mutations in APC or β-catenin with changes in β-catenin levels. Our APC analysis comprised the region from codon 686 to codon 1693 of the APC coding sequence. This includes the mutation cluster region (MCR; codons 1286–1513) which contains 65% of the APC mutations in colorectal tumors (17). We identified an APC mutation in one cell line (DU 4475) that is associated with upregulation of β-catenin. This is the first report of an APC truncation resulting in deregulation of β-catenin in human breast cancer cells. The increased β-catenin levels in DU 4475 is in keeping with the current concept that the truncated APC protein fails to promote β-catenin degradation (8,9). The APC mutation in DU 4475 leads to a profound excess of β-catenin in total cellular extracts (Figure 1A) and cytosolic pools (Figure 2), whereas γ-catenin levels are less affected (Figures 1B and 2).

The APC mutation reported here (E1577stop), has not been described previously (based on the Human Gene Mutation Database, http://www.uwcm.ac.uk/uwcm/mg/hgmd0.html, as of March 6, 2000). It is located at the end of the MCR, in the domain containing seven phosphorylation-dependent β-catenin binding sites and results in a protein lacking the basic region, the microtubule, DLG and EB1 binding domains (7). Although studies have shown that loss of heterozygosity at the APC site on chromosome 5q21 occurs in up to 38% of primary breast cancers (18,19) only two mutations of the APC gene have been reported, both are point mutations leading to amino acid substitutions (18). In addition, an APC mutation in a primary breast cancer has been reported (20), but no documentation of alterations in β-catenin levels was presented.

The DU 4475 cell line was derived from a recurrent thoracic wall tumor following mastectomy for a poorly differentiated invasive ductal breast carcinoma in a postmenopausal patient (21). DU4475 cells are highly transformed and do not adhere to the culture dish, but rather grow in suspension. It is not clear whether upregulation of β-catenin contributes to these growth characteristics. Since APC mutations are rare in breast cancer, we considered the possibility that this mutation was acquired in culture. In our analysis, there was no evidence of a second normal allele. This is interpreted as a hemizygous loss of the other allele since homozygosity for a point mutation would be extremely unlikely. Presumably, the point mutation in one allele (Figure 3B) was complemented by loss of the other allele (Figure 3A). The acquisition of these two independent mutations is unlikely to occur during propagation in vitro; although it cannot be formally excluded. There is no evidence from the original description (21) that the patient had FAP, suggesting a germline APC mutation.

The variability of expression levels of β- and γ-catenin in the other lines may represent the normal range of variation. Alternatively, it may be due to translational and post-translational influences or mutations in other regions of the respective genes. Our results of β- and γ-catenin expression are largely concordant with other reports (11,22). The significance of minor deviations [in contrast to Piercell et al. (11)] we found strong β-catenin expression in BT 474, MCF7, MDA-MB 231, MDA-MB 468 and ZR 75-1] is uncertain. Concurring with our results, a study of 11 breast carcinomas revealed no mutations in exon 3 of the α- or β-catenin genes by RT-PCR and single strand conformation polymorphism (SSCP) analysis (23).

In conclusion, we screened 27 human cell lines derived from breast carcinomas or immortalized breast epithelial cells for alterations in the downstream elements of the Wnt signaling pathway: APC, β- and γ-catenin. We report the first APC mutation with associated β-catenin upregulation in a human breast carcinoma cell line (DU 4475). Unlike colon carcinomas and melanomas, however, deregulation of the Wnt signaling pathway does not seem to be a frequent event in human breast carcinomas.

Acknowledgements

The authors thank Martin Julius, Nick Papadopoulos and Dan Smith for providing comments on the manuscript. This work was supported by grants R01CA75353 and Marilyn Bokemeier Sperry Fund (to J.K.) and R21CA66224 (to L.H.E.)

References


Received January 11, 2000; revised March 22, 2000; accepted March 29, 2000