Reconstitution of TGF-β Sensitivity in the VACO-411 Human Colon Carcinoma Line by Somatic Cell Fusion with MCF-7

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Abstract
We characterized the mechanism of transforming growth factor beta (TGF-β) resistance in the VACO-411 human colon carcinoma line. VACO-411 is unique for several reasons, including having a novel mutator phenotype and wild-type p53. Like many colon tumors, VACO-411 is not growth inhibited by TGF-β. However, VACO-411 represents a subset of colon tumors that are resistant to TGF-β-mediated growth inhibition, despite the expression of functional TGF-β receptors. VACO-411 expresses cell surface TGF-β receptor types I and II, and the coding regions of these receptors are wild type. To further characterize the nature of the VACO-411 defect, we fused VACO-411 with the human breast carcinoma line MCF-7. MCF-7 is also resistant to TGF-β-mediated growth inhibition. However, unlike VACO-411, MCF-7 lacks cell surface expression of TGF-β receptor type II, but does contain an intact postreceptor signaling pathway, as shown by regeneration of TGF-β sensitivity following wild-type TGF-β receptor type II transfection. In contrast to parental VACO-411 and MCF-7, the morphologically distinct cell hybrids were growth inhibited by TGF-β. Therefore, the TGF-β defect in VACO-411 is a postreceptor, loss-of-function mutation which can be genetically complemented. The data suggest that the VACO-411 defect in TGF-β signaling will be able to be further complemented by microcell-mediated chromosome transfer.

The VACO-411 human colon carcinoma line is unique, because it represents a subset of colon tumors with wild-type p53 [7]. Additionally, VACO-411 has a novel attenuated mutator phenotype resulting in increased transversions [2]. This is a nonreplication error phenotype [2]. VACO-411 was derived from the parental VACO-235 adenoma cell line. VACO-411 spontaneously progressed in vivo from tumors of late-passage VACO-235 in nude mice. Like VACO-235, VACO-411 has loss of expression of deleted in colon cancer (DCC) gene, mutant APC protein, mutant K-ras, and wild-type p53 [7]. Additionally, both VACO-235 and VACO-411 have equivalent expression of E-cadherin transcript and Rb and p21 WAF-1 proteins [12]. However, VACO-411 differs from VACO-235 by having acquired resistance to transforming growth factor beta (TGF-β) mediated growth inhibition.
This resistance is through a SMAD4-independent pathway, since both VACO-235 and VACO-411 have a mutant SMAD4, but VACO-235 is still growth inhibited by TGF-β [3]. Karyotypic analysis of VACO-235 and VACO-411 shows the same alterations of chromosomes 1p, 3p, 6q, 7q, 14q, and 18q [7]. The only difference between VACO-235 and VACO-411 is an addition to chromosome 6p in VACO-411 [7]. Therefore, VACO-411 has most likely acquired tumorigenicity and TGF-β resistance through a mechanism such as a point mutation, rather than chromosomal deletion.

VACO-411 is resistant to growth inhibition by TGF-β despite the expression of TGF-β receptors. To further investigate the mechanisms of inactivation of TGF-β receptor type II (RII) signaling in VACO-411, we performed a series of experiments including DNA sequencing, 125I-labeled binding assays, and somatic cell fusion between VACO-411 and MCF-7. MCF-7 cells lack RII, but do contain a functional downstream signaling pathway based upon the results of transfection studies of RII into MCF-7 which restores TGF-β-mediated growth inhibition [10]. Thus, in cell fusions, VACO-411 provides functional TGF-β receptor type I (RI) and RII, while MCF-7 provides an intact postreceptor signaling pathway. Restoration of TGF-β-mediated growth inhibition in the fused cells demonstrates complementation of a loss-of-function mutation in TGF-β signal transduction. Alternatively, failure to restore TGF-β-mediated growth inhibition in the fused cells indicates activation of a dominant inhibitor of the TGF-β signaling pathway. Further characterization of the VACO-411 TGF-β signaling defect is of interest because of the current use of this unique cell line in diverse studies including DNA repair mechanisms, chemotherapeutic drug effects, and TGF-β signaling.

Materials and Methods

Cell Culture

VACO-411 was transfected with puromycin resistance gene using the pBABE-puro vector and calcium phosphate, and MCF-7 was transfected with neomycin resistance gene [10]. The VACO-411 cell line has been previously characterized [7]. MCF-7 (clone 20) was maintained in 500 μg/ml neomycin (G418). MCF-7 and VACO-411 were maintained in 0.5 μg/ml puromycin, and MCF-7 was added to one of the wells 14 h later. After 2 weeks, colonies were stained with methylene blue, and colonies containing >50 cells were scored as positive.

Sequence Analysis of RI and RII

Total RNA was isolated from VACO-411 by centrifugation through a cesium cushion, and cDNA was made using random hexamers, 1× AMV buffer (Boehringer Mannheim), and 0.5 mM dNTPs. RI was PCR amplified using 0.125 mM dNTPs, 1× buffer (Boehringer Mannheim), and 1 U Taq polymerase (Boehringer Mannheim) for 35 cycles consisting of 95°C for 30 s, 55°C for 1 min, and 70°C for 2 min. Primers were as follows: P1 sense: 5'-ATGGAGGCGCGGTGCTGCTCGCGG-3'; P2 sense: 5'-CATGGCCGCAGCTGCTATTGCTGACGACG-3'; P3 sense: 5'-GTTGCGCTTTAAAGATCTTCTCTAGTGA-3'; P4 sense: 5'-TACAGACTGGCGAGTACATGATTCTCCTCTAG-3'; P5 antisense: 5'-GATATAGACCATACACATGAGTGAGATGC-3'; P6 antisense: 5'-CGTAACATTACAGTTGTGATAAAATCTCTG-3'; P7 antisense: 5'-GGAGCAATATCATAAGTGATCTCTGAGGC-3'; P8 antisense: 5'-CTCAGTGGAGTGAAACTCTGACCCTGCCC-3'; RI PCR products were directly sequenced using the Sequenase kit (Amersham). Additionally, RI PCR products were cloned into the TA vector (Invitrogen), and DNA from pooled clones was sequenced using the Sequenase kit. RII primers were as follows: 297 sense: 5'-CGCTGGGGGCTCGGCTGCTCCGCG-3'; P66 sense: 5'-TGCGCTTTAGCTGCTGAGTCGCCG-3'; RI PCR products were cloned into the TA vector (Invitrogen), and DNA from pooled clones was sequenced using the Sequenase kit.

Cross-Linking

Receptor cross-linking was performed [10]. TGF-β1 was iodinated by the chloramine-T method. Confluent monolayer cultures of MCF-7 or VACO-411 were incubated with 200 μM of 125I-TGF-β or in the presence of 20 μM cold TGF-β1 for 3 h at 4°C. The receptor-bound 125I-TGF-β1 was cross-linked with disuccinimidyl suberate. Labeled cell monolayers were solubilized in 1% Triton X-100 containing 1 mM phenylmethylsulfonyl fluoride. Equal amounts of cell lysate protein were electrophoresed in 4–10% gradient SDS-PAGE under reducing conditions and exposed for autoradiography.

Cell Fusions

VACO-411 (1 × 10⁶ cells) was plated per each well of a 6-well plate and allowed to attach for 2 h. MCF-7 (1 × 10⁶ cells) was then added to each well of VACO-411 and plated in a 37°C, 5% CO₂ incubator overnight. The cells were fused by adding 1 ml of 48% polyethylene glycol 1500 (BDH) per well for 60 s. Cells were then rinsed and fed with supplemental McCoy's medium containing 10% fetal bovine serum. Fusions were performed in triplicate. The next day, each well was split to four 100-mm plates, each plate containing 5 ml of supplemental McCoy's containing 10% fetal bovine serum.

Measure of TGF-β Responsiveness

For cell counts of VACO-411 and MCF-7, 20,000 cells were plated in 1 well of a 24-well plate. TGF-β1 (20 ng/ml) was added 14–16 h later. Viable cells were counted 5 days later by hemocytometer. For clonogenic assays of VACO-411 × MCF-7 whole-cell fusions, hybrid colonies formed in the absence of TGF-β were pooled and divided into 2 wells of a 24-well plate. TGF-β1 (20 ng/ml) was added to one of the wells 14 h later. After 2 weeks, colonies were stained with methylene blue, and colonies containing >50 cells were scored as positive.

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