Frizzled Activation by Wnt-1 Is Required for β-Catenin-T Cell Factor Dependent Transcription in Esophageal Cancer

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Abstract: Although, accumulation of nuclear and cytoplasmic β-catenin has been observed in ESCCs, mutation of APC and β-catenin are not found in ESCCs. Therefore, another mechanism for cytoplasmic β-catenin accumulation might exist in ESCCs. Materials and Methods: Human ESCCs cell lines and the 293 stable transfectants expressing Wnt-1, Wnt-5A, and Wnt-7A were cultured under standard conditions. The TOPFlash or FOPFlash reporter plasmids were transfected. Results: Transfected mutant β-catenin as well as an axin fragment harboring the GSK3β interaction domain, the latter a potent GSK3β inhibitor, both robustly activated pTOPFlash in ESCCs cells. When pTOPFlash/pFOPFlash reporters were transfected in ESCCs cell lines followed by cocultivation with 293 cells that stably express Wnt-1, all cell lines except one demonstrated TCF mediated transcriptional. But, cells were co-cultured, Wnt-7A or Wnt-5A did not activate TCF mediated transcription in a cell number dependent fashion. Discussion: We report the activation of TCF promoter gene by external Wnt stimuli in ESCCs cells.

Key words: esophageal carcinoma, Wnt, TCF, β-catenin

Introduction

Human esophageal cancer is often an aggressive tumor with a poor prognosis (1). Recently, accumulation of nuclear and cytoplasmic β-catenin has been observed in esophageal squamous cell carcinoma (ESCC) (2, 3). It is involved in Wnt-Frizzled pathways that regulate cellular differentiation and proliferation (4, 5). In the absence of growth or differentiation signals, cytoplasmic β-catenin is low, since the protein is sequestered in complexes, which include the adenomatous polyposis coli (APC) protein, a
Wnt-1 activate TCF transcription

Materials and Methods

Plasmids

pGL3-OT (pTOPFLASH) and pGL3-OF (pFOPFLASH) (gift of Dr. Bert Vogelstein) are luciferase reporter constructs containing wild type and mutated trimeric TCF binding sites, respectively (4). pClneo-β-catenin XL and pClneo-β-catenin Δ45 are expression constructs for wild-type and constitutively active mutant form of β-catenin, respectively (gift of Dr. Bert Vogelstein) (18). pCS2MT-axin GID1-2 and pCS2MT-axin GID L/P are constructs which express wild type and mutated GSK-3β interaction domains of Xenopus laevis axin, respectively (gift of Dr. Peter S. Klein) (19).

Cells

Human esophageal squamous cell carcinoma cell lines, TE1, TE3, TE5, TE8, TE10, TE11, TE12, and TE15, embryonic kidney 293 cells, and the 293 stable transfectants expressing Wnt-1, Wnt-5A, and Wnt-7A (gift of Dr. Elizabeth Wilder) were cultured under standard conditions, namely at 37°C under 5% CO₂ in Dulbecco's modified Eagle's medium (Life Technologies, Grand Island, NY) supplemented with 10% fetal calf serum (Sigma, St. Louis, MO), and 100 units/ml penicillin, and 100 μg/ml streptomycin (Life Technologies, Grand Island, NY).

Transfections and Luciferase Assay

Transfections were performed by using LipofectAMINE (Life Technologies, Inc., Grand Island, NY) according to the manufacturer's instructions. At 24 h prior to transfection, 2.5 x 10⁵ cells were seeded in 12-well plates. To assay the ability of Wnt to activate TCF/LEF transcription, TE series and EPC2 cells were transfected with 0.25 μg of the TOPFlash or FOPFlash reporter plasmid. At 36 hours after transfection, cells were harvested for luciferase
activity. All test variables were performed in triplicate. Luciferase assays were performed by using the Luciferase Assay System (Promega, Madison, WI) in Microtiter® Plate Lumino-meter (DYN-EX Technology, Chantilly, VA). β-galactosidase activities were determined by standard methods as a control for transfection efficiency.

Results

TCF mediated transcription is inactive in ESCC cell lines.

To determine TCF mediated transcriptional activity, we transfected pTOPFLASH and pFOPFLASH luciferase reporter constructs in several ESCC cell lines. None of the ESCC cell lines tested showed any appreciable TCF mediated transcriptional activity in the absence of external Wnt stimuli. The lack of constitutive activation of TCF mediated transcription in ESCC cell lines suggested that these cell lines are likely to carry intact genes for β-catenin, APC or axin whose mutations are frequently observed in certain subsets of cancers with activated β-catenin dependent TCF mediated transcription.

To exclude the possibility that ESCC cell lines lack the necessary transcriptional machinery including TCF transcription factors, we ectopically expressed several proteins known to potently activate β-catenin dependent TCF-mediated transcription. As shown in Fig. 1, transfected mutant β-catenin as well as an axin fragment harboring the GSK3β interaction domain, the latter a potent GSK3β inhibitor, both robustly activated pTOPFLASH in TE8 cells.

Figure 1. β-catenin-Tcf dependent transcriptional activity in TE8 cells.

We investigated whether the machinery of Wnt-GSK-3β-Tcf/LEF pathway were present or not in ESCC cells. We transfected green fluorescent protein (GFP), empty vector (pCI neo), wild type β-catenin, mutant β-catenin, and wild type axin (Axin GID L/P) or mutant axin (Axin-GID) to TE8 cells. GFP and pCI neo served as transfection controls. Transfection of mutant β-catenin or mutant axin activated TCF/LEF reporter gene compared to wild type. These results indicated that ESCC cell had the complete machinery of the pathway.

Regulation of TCF mediated transcriptional activity by Wnt-1 in ESCC cell lines.

To investigate the possible activation of Wnt signaling pathway in ESCC cell lines, we tested the effect of Wnt-1, Wnt-5A, and Wnt-7A on TCF mediated transcriptional activity. When pTOPFLASH/pFOPFLASH reporters were transfected in ESCC cell lines followed by co-cultivation with 293 cells that stably express Wnt-1, all cell lines demonstrated TCF mediated transcriptional except TE12 (Fig. 2). Transcriptional activation was not observed when the transfected cells were co-cultured with parental 293 cells. Therefore, the activated TCF mediated transcription in ESCC cell lines is attributable to the expressed Wnt-1 in the medium. Consistent with this idea, TCF mediated transcription in TE8 cells was activated depending on the cell number of the co-cultured stable transfectants (Fig. 3). When TE8 cells were co-cultured, Wnt-7A but not Wnt-5A weakly activated TCF mediated transcription in a cell number dependent fashion (Fig. 3).
Wnt-1 activate TCF transcription

Discussion

We report the activation of TCF promoter gene by external Wnt stimuli in ESCCs cells. The Wnt/Frizzled signaling pathway stabilizes β-catenin/APC complexes and increases free intracellular pools of β-catenin by inactivation of GSK-3 (7). We showed that Wnt-1 activated the TCF promoter gene. To our knowledge, this is the first report of functional TCF/LEF activation by Wnt-1. Our results indicate the possibility that Wnt has potential role in development and progression in ESCC. Inactivation of APC function induces stabilization of the β-catenin protein. More recently, the stabilized free β-catenin has been found to bind to high mobility group box-containing transcription factors including LEF (6) and TCF (4) and the complexes translocate to the nucleus. The nuclear β-catenin is thought to act in conjunction with the LEF/TCF transcription factors to activate gene expression associated with cell proliferation or inhibition of apoptosis (20).

Activated Frizzled receptors induce the stabilization of the cytoplasmic component β-catenin by blocking the function of a multiprotein β-catenin destruction complex (21). This complex consists of the scaffolding component axin, or the related conductin. These bind to the tumor suppressor protein APC, GSK3β and β-catenin through separate domains. In the absence of a Wnt signal, GSK3 mediates the phosphorylation of β-catenin and the ubiquitination machinery, and finally degraded in proteasomes. In the presence of Wnt, the cytoplasmic phospho-protein dishevelled is activated and interferes with the β-catenin destruction complex. GSK3 activity is inhibited, and the phosphorylation of β-catenin is blocked. Hypophosphorylated β-catenin is no longer degraded and accumulates.
in the cytoplasm and the nucleus.

Wnt-1 was identified as a preferred integration site for mouse mammary tumor virus in breast adenocarcinoma (22). In addition, Wnt-1 is able to transform cultured mammary epithelial cell lines (22) and transgenic mice expressing Wnt-1 in the mammary gland develop mammary epithelia hyperplasias and adenocarcinomas (23). However, in these transgenic studies, it is clear that Wnt-1 expression alone is insufficient for malignant (24).

A long-standing difficulty in studying the action of mammalian Wnt genes has been the inability to isolate their products in the soluble form that demonstrates functional activity (25). Our results indicate that Wnt-1 protein can act on target cells externally as a soluble secreted factor.

Reference


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