Claudin6 Suppresses Migration and Invasion via Blocking Smads/Snail/MMP2/9 Pathway in MCF-7 and SKBR-3 Cell Lines

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Abstract

Background: We have previously reported that CLDN6-mediated SB431542 suppresses EMT, migration and invasion with SB431542, The cells were incubated with MMP2/9 Inhibitor I selective antagonists of MMP-2/MMP-9. 2- Snail was knocked down by shRNA in MCF-7 and SKBR-3 cell lines. The levels of MMP2 and MMP9 were examined by RT-PCR and Western blot. The expression of E-cadherin and N-cadherin were analyzed by Western blot and Immunofuorescence Microscopy. Vimentin expression was detected by Western blot. Migration and invasion were analyzed by Wound Healing Assay and Matrigel Invasion Assay.

Results: MMP2/9 Inhibitor I reversed the efects of Knocking down CLDN6 on downregulation of E-cadherin, up-regulation of N-cadherin and Vimentin, facilitation of migration and invasion. In MCF-7-shSnail and SKBR-3-shSnail cells, migration and invasion were inhibited, E-cadherin was up regulated, MMP2, MMP9, N-cadherin and Vimentin were downregulated.

Conclusions: The experiment demonstrates that Claudin6 suppresses epithelial-mesenchymal transition, migration and invasion via blocking SMADs/Snail/MMP2/9 pathway in MCF-7 and SKBR-3 cell lines.

Keywords: CLDN6; Snail; MMP2; MMP9; SMADs

Introduction

Breast cancer is a main cause of cancer-associated death in women [1]. Recurrence and metastasis are the primary reasons for breast cancer-associated patient death [2]. CLDN6 is a member of the claudins family, which plays an important role in the biology of many neoplastic diseases. Its effects are often tissue specific [3-6]. EMT is one of the most important steps during cancer progression [7,8]. Cancer cells grow in situ initially. Following loss of polarity and changes in morphology, the malignant cells exit from their place of origin and invade adjacent local tissue or metastasize into distant organs through the blood vessels and continue to grow [9,10]. The epithelial markers are downregulated and the interstitial markers are upregulated [11]. The process of EMT is regulated by EMT associated transcription factors Twist, Snail, Zeb1 [12-17], and signaling pathways including TGF-β and Wnt pathway [18]. In our previous experiments, it is found that CLDN6-mediated SB blocked EMT, invasion and migration [19].

Snail is a zinc Finger structure transcription factor, one of the key regulators that promotes EMT, migration and invasion in many types of malignant tumors including head and neck squamous cell carcinoma (HNSCC) [20], lung cancer [21], hepatocellular carcinoma [22], Snail can regulate the expression of Matrix Matalloproteinases (MMPs) in numerous cancers [23-25]. MMPs degrade the extracellular matrix (ECM) to facilitate the metastatic process [26].

Our previous experiment demonstrated that CLDN6-mediated SB inhibited invasion, migration and EMT in breast cancer cells, as well as the downregulation of Snail, MMP2 and MMP9 [19,27]. In our present experiments, we have verified the hypothesis that CLDN6-mediated SB inhibits invasion, migration, and EMT via down regulation of snail/MMP2/9 in MCF-7 and SKBR-3 cell lines.

Transient transfection with short hairpin RNA

Transient transfection CLDN6: Cells were transfected with short hairpin RNA and Lipofectamine 2000 (Invitrogen), following the instructions. shRNA targeting CLDN6 (5'-GTGCAAGGTGTACGACTCA-3') and a negative control shRNA were obtained from Shanghai GeneChem Co. Ltd.

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Transient transfection snail: Following the method of Ding et al. [28], shRNA (containing sense and antisense sequences linked by a hairpin loop: TTCAAGAGA) was designed and constructed into the vector pGCsilencer TM U6/Neo/GFP (GeneChem, China). The MCF-7 and SKBR-3 cells were then transfected with SuperFect®. Transfection Reagent (QIAGEN, USA). The negative control cell line was generated by treating cells with the vector pGCsilencer TM U6/Neo/GFP constructed with oligonucleotides, which have no homology to the human gene. The control group was the untreated MCF-7 and SKBR-3cell lines.

Reverse transcription-polymerase chain reaction (RT-PCR)

Reverse transcription-polymerase chain reactions were carried out as described previously. Total RNA was extracted from the cloned cells using TRIzol (Invitrogen, USA) following the manufacturer's instructions. One microgram of total RNA was reverse-transcribed to synthesize cDNA using the MMuLVreverse transcriptase (TaKaRa, Japan) for 60 min at 42 , 0.5ug cDNA was used for PCR. SNAIL, MMP2 and MMP9 were amplified along with GAPDH as an endogenous control following the instructions of Premix LA Taq Kit (TaKaRa, Japan). The PCR reaction conditions and the primer sequences are listed in Table 1. After electrophoresis, the gel was captured and analyzed by an imaging system (Syngene, Cambridge, UK).

Western blotting analysis

Western blotting analyses were carried out as described previously [29]. Anti-MMP2, MMP9, N-cadherin, and Vimentin antibodies were obtained from Abcam (Cambridge, UK), an anti-E-cadherin antibody was purchased from Bioworld Technology (Dublin, OH, USA), the anti-β-actin antibody was from Santa Cruz (Santa Cruz, CA, USA). The blots were imaged and analyzed using an ECL Western blotting system (GE, Fairfield, CT, USA).

Immuno uorescence microscopy

An immunofluorescence assay was carried out to evaluate the expression of these targets as previously described [30]. Cells were cultured with primary antibodies against E-cadherin diluted to 1:400 and N-cadherin diluted at 1:250 at 4

by Snail in tumors [36-39]. However, this is in contrast to the results of

tight junction assembly during epithelial differentiation. Ann N Y Acad Sci 1397:80-99.

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