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Research Article

Expression of WNT genes in cervical cancer-derived cells: Implication of WNT7A in cell proliferation and migration

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ABSTRACT

According to the multifactorial model of cervical cancer (CC) causation, it is now recognized that other modifications, in addition to Human papillomavirus (HPV) infection, are necessary for the development of this neoplasia. Among these, it has been proposed that a dysregulation of the WNT pathway might favor malignant progression of HPV-immortalized keratinocytes. The aim of this study was to identify components of the WNT pathway differentially expressed in CC vs. non-tumorigenic, but immortalized human keratinocytes. Interestingly, WNT7A expression was found strongly downregulated in cell lines and biopsies derived from CC. Restoration of WNT7A in CC-derived cell lines using a lentiviral gene delivery system or after adding a recombinant human protein decreases cell proliferation. Likewise, WNT7A silencing in non-tumorigenic cells markedly accelerates proliferation. Decreased WNT7A expression was due to hypermethylation at particular CpG sites. To our knowledge, this is the first study reporting reduced WNT7A levels in CC-derived cells and that ectopic WNT7A restoration negatively affects cell proliferation and migration.

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Introduction

Cervical cancer (CC) is one of the most common malignant tumors in women worldwide [1]. Clinical, molecular, and epidemiological data have identified High-risk Human papillomaviruses (HR-HPV) as an etiological agent that is necessary, but not sufficient, in the development of this tumor [2–4]. In 2005, it was proposed that the WNT pathway might be implicated in the multi-step progression of HPV-immortalized keratinocytes towards malignancy [5]. The WNT signaling pathway is a complex network of proteins involved in cell differentiation, proliferation, migration, and cell polarity, processes that are also important during embryonic development, tissue regeneration, stem cell maintenance, and homeostasis [6].

WNT ligands are secreted glycoproteins that bind to transmembrane receptors denominated Frizzled (FZD) and to co-receptors LRP5 or LRP6 (from Low-density lipoprotein Receptor-related protein 5/6). In humans, the complexity of the WNT pathway is conferred by possible heterodimers formed with 19 different WNT ligands, 10 FZD receptors, 2 LRP co-receptors, and a few non-FZD receptors, such as tyrosine kinase proteins (ROR and RYK) [6].

Currently, there are at least three WNT signaling pathways described: the canonical pathway, also known as WNT-β-catenin; the calcium-dependent pathway (WNT/Ca^{+2}), and the Planar cell polarity pathway (PCP). The best-studied of these is the canonical pathway, which involves the inhibition of Glycogen synthase kinase-3β (GSK-3β), which in turn blocks β-catenin degradation in the cytosol, resulting in its accumulation and subsequent migration to the nucleus, where it forms transcription complexes with LEF/TCF, proteins that lead to the activation of several genes [7]. The canonical pathway has been linked with other signaling pathways, such as the fibroblast growth factor (FGF) and transforming growth factor beta (TGF-β) signaling networks [8].

The non-canonical WNT signaling pathways are independent of β-catenin and were initially related with the modulation of cell movement [9]. Activation of the non-canonical WNT/Ca^{2+} pathway causes the intracellular flow of calcium ions, which leads to the activation of Ca^{2+}-dependent effector molecules, such as Calcium/calmodulin-dependent Kinase II (CamKII), the Nuclear factor of activated T cells (NFAT), and Protein kinase C (PKC). NFAT is associated with the regulation of several genes, such as those for cytokines, which in turn regulate cell cycle, differentiation, and apoptosis [7]. WNT pathway ligands have been classified into two groups: those that can induce the canonical pathway, and those that can activate the non-canonical pathways. Aberrant expression of WNT ligands has been implicated in numerous types of cancer, not only in leukemia, breast, gastrointestinal, and lung [10–13], but also in CC [14].

WNT7A is a 39 kDa glycoprotein of this family, and has been strongly implicated in female reproductive tract development, as it has been proven that knockout mice for this gene have impaired sexual organogenesis [15]. WNT7A expression possesses controversial roles in different types of cancer; in ovarian cancer, endometrial carcinoma, and human malignant pleural mesothelioma, its over- or underexpression has been proposed as a prognostic marker [16–18]. On the other hand, reconstitution of WNT7A activity in lung cancer and in leukemic cells demonstrated an inhibitory role in cell proliferation [19,20] but, at present, there is no report to our knowledge on the relationship between WNT7A activity and CC.

Although recent investigations have made it evident that the activity of the different ligands, whether canonical or non-canonical, might be influenced mainly by the cell context of the receptors, inhibitors, and other ligands, the WNT network continues to be a complex subject of research.

In this study, we investigated the expression of key components of WNT pathways to determine expression profile changes between the immortalized non-tumorigenic keratinocyte cell line HaCaT and the tumorigenic CC cell lines HeLa and SiHa. We focused our attention especially on WNT7A, in which its role in proliferation and migration was explored. Our results indicate that expression of WNT7A plays a major role in the regulation of normal keratinocyte proliferation; thus, inhibition of WNT7A expression appears to comprise an important step during cervical carcinogenesis. These findings could be important for generating further prognosis/diagnosis tools, or even for anticancer therapies.

Materials and methods

Cell culture

Cervical carcinoma-derived cell lines HeLa, SiHa, and C33-A (obtained from ATV-DKFZ, Heidelberg, Germany) and the non-tumorigenic human epithelial cell line HaCaT, kindly obtained from Dr. Boukamp, DKFZ, Heidelberg, Germany [21] were grown in Dulbecco's modified Eagle's medium (DMEM) containing GlutaMAX™, 10% Fetal bovine serum (FBS), 100 U/ml Penicillin, and 100 μg/ml Streptomycin (all from GIBCO®, Life Technologies Corporation, Carlsbad, CA, USA). Primary keratinocytes (Ker) were obtained from newborn foreskin; briefly, the tissue was first treated with antibiotics in growth media for 24 h at 37 °C, fatty and connective tissue was then removed, and the tissue was finely chopped (<1 mm) and treated with 25 U/ml collagenase (Sigma-Aldrich, México) and 25 U/ml dispase (GIBCO®, Life Technologies Corporation) overnight at 4 °C to allow epidermal tissue separation. Afterward, epidermal tissue was stirred for 30 min at 37 °C in 2 ml of 0.25% trypsin–EDTA solution (from GIBCO®, Life Technologies Corporation). After trypsin neutralization with media containing 10% FBS, cells were collected by centrifugation and later resuspended in 13 ml of defined keratinocyte serum-free medium supplemented with pituitary gland extract, Epidermal growth factor (EGF), and antibiotics (all from GIBCO®, Life Technologies Corporation). Cells were then cultured in flasks pre-treated overnight with FBS, the latter to achieve more efficient cell adhesion. All cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2.

Cervical samples

Cervical samples were collected with a cytobrush during gynecological examination through colposcopy. If no cervical lesions were found, cells were collected only by cytobrush, placed into the transport medium (PreservCyt® solution; Hologic, Bedford, MA, USA), and processed immediately for DNA and RNA extraction. If evident CC was observed, biopsies for histopathology testing and RNA extraction were additionally taken. Only samples with Squamous cell carcinoma (SCC) confirmation were included in this study. Cervical samples without lesions (controls) and without HPV infection were included.