

## **The Expression of NDRG1, VEGF and Ki-67 in Condyloma Acuminatum**

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### **ABSTRACT**

**Objective:** To explore the expression and significance of NDRG1, VEGF and Ki-67 in lesions of CA (Condyloma Acuminatum). **Methods:** SP immuno histochemistry method was used to measure the expression of NDRG1, VEGF and Ki-67 in 48 cases of CA and 18 normal skin controls. **Results:** The positive rates of NDRG1, VEGF and Ki-67 were 63.83% (40/48), 93.75% (45/48) and 85.42% (41/48) in CA tissues, meanwhile 27.78% (5/18), 94.44% (17/18) and 61.11% (11/18) in the controls. The intensity of expression of NDRG1, VEGF and Ki-67 in CA tissues was obviously higher (++ ~ ++++) than that in normal controls (- ~ ++). There was a significant difference both in the positive rates and the expression intensity of NDRG1, VEGF and Ki-67 between the two groups ( $P < 0.05$ ). The Spearman rank correlation analysis showed that, the expression of NDRG1 protein and VEGF protein were positively correlated by the Spearman rank correlation analysis ( $r = 0.346$ ,  $P = 0.016$ ). **Conclusion:** The higher expression of NDRG1 and VEGF in CA tissues, they influenced both the occurrence and development of CA together.

**Keywords:** *condyloma acuminatum, NDRG1, VEGF, Ki-67*

### **INTRODUCTION\***

Genital warts (condyloma acuminatum, CA) is made by human papilloma virus (human papilloma virus comes, HPV) infection caused by a common sexually transmitted diseases. Condyloma acuminatum (CA) is the infection of human papilloma virus (HPV), and become one of the most common sexually transmitted disease[1]. CA growth fast, easy to relapse after treatment, and there is a close relationship with the incidence of genital cancer, cause high attention. NDRG1 gene was found in recent years, and the new genes was associated with cell differentiation, and closely related to the tumor occurrence, development and metastasis. It can be a variety of differentiated regulator inducing expression[2]. VEGF is the strongest of the current known, the most specific vascular regulating factor[3]. Ki-67 antigen is

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one of the most widely used cell proliferation marker, and accurately reflects the activity of cell proliferation. It lacks of system research of NDRG1, expression of VEGF and Ki-67 in CA tissue. This study applies the NDRG1 and VEGF immunohistochemical SP method and Ki-67 expression in CA tissue, and investigates the relationship between their expression, and the possible function in the onset of CA.

### **MATERIAL & METHOD**

#### **Case Information**

48 patients, from August 2009 to June 2010, with CA were from our hospital skin venereal outpatient service. All cases with typical clinical manifestations, and clear diagnosis for the histopathologic examination. Male 29 cases, female 19 cases; 18 ~ 56 years, average age  $24.15 \pm 7.58$  years old; Course of 16 ~ 120 days, an average of  $51.29 \pm 3.23$  days. 18 samples of normal male circumcision tissue were from our

hospital urology clinic, age: 18~28 years old, average age: 22, 32 years.

### **Main Reagent**

Sheep polyclonal antibody NDRG1 is provided by the Santa Cruz companies in the United States; Mouse anti human VEGF, Ki-67 ready-to-use SP detection kits, DAB enzyme substrates are purchased fujian agent new biological engineering co., LTD.

## **EXPERIMENTAL**

### **NDRG1, VEGF and Ki - 67 expression by SP Method**

According to mildew avidin Peroxidase labeled chain (Streptav idin/Peroxidase, SP) staining kit steps, all slice staining was under the same conditions, each batch dyeing have positive control and negative control. Positive control was with a known positive biopsy, negative control was incubation biopsy with PBS instead of a fight.

### **Immunohistochemical**

Staining Results NDRG1 positive color are tan particles, mainly for the cytoplasmic staining; VEGF positive color are tan particles, mainly for the cytoplasm staining; Ki-67 positive color tan particles as the nucleus. Grading standard reference literature[4], observed at

high magnification, and each view to observe cell number not less than 200, according to the percentage of positive cells in a tissue section and half quantitative classification of tinting strength score: (1) positive cell percentage 10% or less for 0 score, 11% ~ 25% for 1 score, 26% ~ 50% for 2 score, 51% ~ 75% for 3 score, 76% or more for 4 score; (2) the tinting strength: 0 score, no color; 1 score, light yellow; 2 score, yellow; 3 score, tan. Take the above (1) and (2) the product of the two points as the total points: 0 score is for negative (-), 1~4 score for weakly positive (+), 6~8 sore for the positive (++), 9~12 score strong positive (+++).

### **Statistical Processing**

Using SPSS17.0 statistical software package to deal with the data obtained,  $\chi^2$  test and Spearman correlation test analysis.

## **RESULTS**

### **The Expression of NDRG1 in CA and Normal Foreskin Tissue**

40 cases in 48 cases of CA tissue were with the NDRG1 positive expression, the positive expression rate was 83.33% (40/48), the positive cell shading is yellow to tan particles, its expression intensity in ++ ~ +++, and was mainly expressed in the cytoplasm of cells or cell membrane, but mainly in a cell cytoplasm,

**Table 1:** NDRG1 expression in CA tissue and control group tissue

	Case Qty	NDRG1				Positive rate (%)	$\chi^2$	P
		-	+	++	+++			
CA Group	48	8	5	16	19	83.33	61.241	<0.05
Control Group	18	13	2	2	1	27.78		

**Table 2:** VEGF expression in CA tissue and normal control group tissue

	Case Qty	VEGF				Positive (%)	Rate	$\chi^2$	P
		-	+	++	+++				
CA Group	48	3	5	22	18	93.75	0.00		>0.05
Control Group	18	1	9	7	1	94.44			

**Table 3:** The correlation analysis between NDRG1 and VEGF expression in CA tissue

NDRG1	VEGF		<i>r</i>	<i>p</i>
	+	-		
+	39	1	0.346	0.016
-	6	2		

with the focal or diffuse distribution(see Fig 1). In the control group, for 18 cases, 5 cases of NDRG1 positive expression expression, with rate 27.78% (5/18), and the intensity of expression was - ~ ++ (see Fig 2). Two groups of positive expression rate and positive expression intensity were statistically significant (see chart 1).

#### ***VEGF Expression in CA tissue and Normal Foreskin Tissue***

48 cases with 45 cases of VEGF positive expression in CA tissue, mainly expressed in the cytoplasm of CA tissues cells, the positive expression rate was 93.75% (45/48), expression strength mainly at ++ ~ +++, for positive cells shaded yellow, tan particles, positive cells spreaded in skin all layers except the corneous layer, but expression was obvious in the base layer, the stratum spinosum layer (see Fig 3). In control group, VEGF positive expression rate was 94.44% (17/18), expressing intensity in + ~ ++, (see Fig 4). VEGF expression positive rate of the differences in the two groups has no statistical significance, but the strength intensity of positive expression between the two groups was statistically significant(see table 2).

#### ***Ki-67 Expression in CA Tissue and normal Epithelium***

48 cases of CA Tissue, there were 41 cases were Ki-67 positive, the positive expression rate of 85.42% (41/48), expression strength mainly in ++ ~ +++, the positive cell nuclei positioning coloring, tan grains, positive cells located throughout the base layer and the lower part of stratum spinosum, and small expression were mainly in stratum spinosum upper and particles in the layer (see Fig 5). The positive rate in normal control group were 61.11% (11/18), the

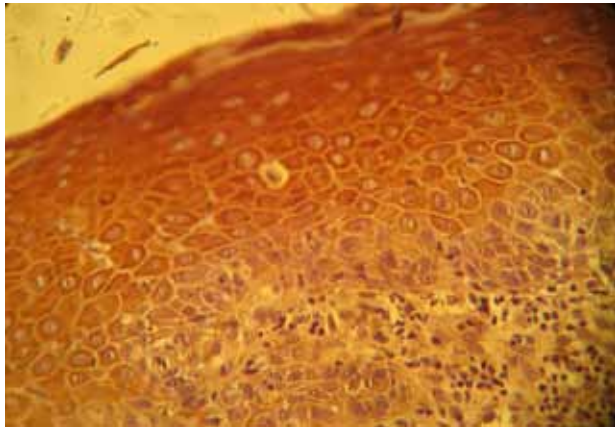
intensity of expression was in - ~ +, mainly was observed in the underlying cells (see Fig 6); The positive comparison difference was statistically significant ( $p < 0.05$ ), the intensity of positive expression of similarity between the two groups have statistical significance ( $p < 0.01$ ).

#### ***The Correlation Analysis between NDRG1 and VEGF Expression in CA Tissue***

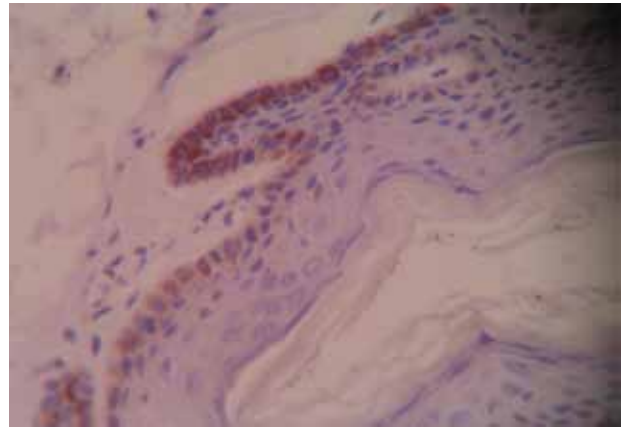
In 40 cases of NDRG1 protein positive, 39 cases of VEGF were with protein positive, and 1 case of VEGF was with protein negative; In 45 cases of VEGF protein positive, 39 cases of NDRG1 were with protein positive, 2 cases of NDRG1 were with protein negative. Both correlation, through Spearman rank correlation analysis, were found that  $r = 0.346$ ,  $p = 0.016$ , indicate that positive correlation of NDRG1 protein between the expression of VEGF protein (see table 3).

### **DISCUSSION**

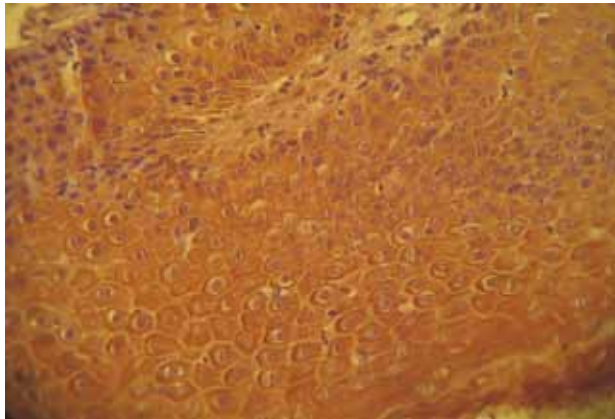
NDRG family, also named N - Myc downstream regulated gene familily, as a new set of genes, had been discovered recently. Their amino acid homology is very close, but the distribution and function vary widely in human body tissue[5,6]. NDRG1 were the first gene to be named and cloned, the gene involved in the process of cell differentiation, proliferation and apoptosis[7,8]. NDRG1 significantly reduced the expression of VEGF, IL-8 and MMP-9 (matrix metalloproteinase-9), and inhibited angiogenesis, through p53 and one absent gene of PTEN (phosphate and tension homology delated on chromosome 10). So, NDRG1 achieved the purpose of inhibiting tumor growth and metastasis by influencing the tumor cell surface molecules and its surrounding tissues



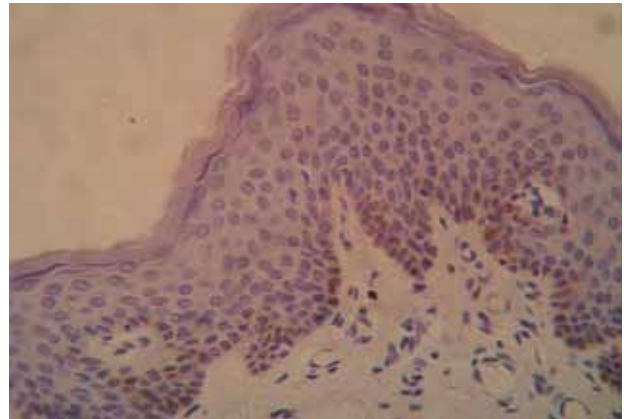
**Fig 1.** NDRG1 Expression in CA Tissue DAB Chromogenic, Immunohistochemical SPx400



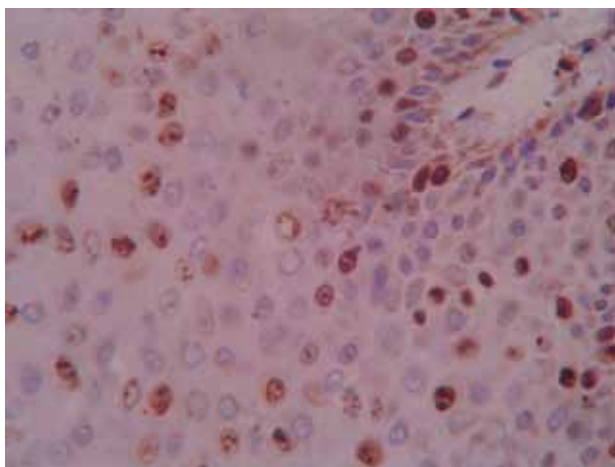
**Fig 2.** NDRG1 Expression in Normal Foreskin Tissue, DAB Chromogenic, Immunohistochemical SPx400



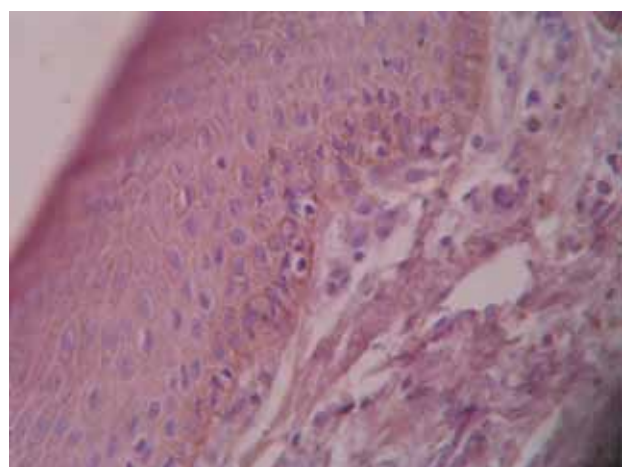
**Fig 3.** VEGF Expression in CA Tissue DAB Chromogenic, Immunohistochemical SPx400



**Fig 4.** VEGF Expression in Normal Foreskin Tissue, DAB Chromogenic, Immunohistochemical SPx400



**Fig 5.** Ki-67 Expression in CA Tissue DAB chromogenic, SPx400



**Fig 6.** Ki-67 Expression in Normal Tissue DAB chromogenic, SPx400

of the formation of blood vessels. This study used immunohistochemical method to detect NDRG1 expression in CA tissue. The results showed NDRG1 positive expression rate in the CA tissue was higher than the normal control group. The difference was with statistically significant, the intensity of positive express was also statistically significant, consistent with the literature[9,10]. NDRG1 in CA might affect the CA angiogenesis and incidence.

VEGF was the glycoprotein, which had been founded in 1989 by Ferrarra [11] purification. It was the key growth factor to promote angiogenesis, VEGF was found to be the strongest and most specific angiogenic factors to promote the growth of endothelial cells and inducing angiogenesis. We applied the SP method to detect VEGF expression in CA tissue. The results showed that: VEGF has high positive expression in the CA tissue cells, with positive expression rate 93.75% (45/48), with the expression strength of ++ ~ ++++. While, in normal foreskin tissue, the positive expression rate was 94.44% (17/18). Both the positive expression rate have no statistical significance, but the intensity difference of both positive expression were statistically significant, and the positive expression intensity of VEGF in CA group was obviously higher than that of control group. The expression in CA tissue may involve in the CA tissue hyperplasia, through a certain forms of angiogenesis.

Ki-67 antigen was a DNA binding protein, with molecular weight of 359 kd and 320 kd, gene location on chromosome 10, composed of 15 exons transcription processing, existed in proliferating cells[12]. Its expression was closely related to cell cycle, through its phosphorylation, phosphorylation to affect the cell cycle, and could accurately control the cell cycle. Ki-67 monoclonal antibody could mark the cell nucleus antigen of the late G1, S phase and G2 and M phase, while, the early G0 and G1 nucleus was not marked. It had

been considered one of the most ideal index of cell proliferation activity, because of the short half-life, cell proliferation cycle from rapid degradation, long half-life was obviously better than the reliability of proliferating cell nucleus antigen (PCNA), could fully reflect the proliferation of cell proliferation. This study found that Ki-67 positive expression rate and expression intensity in CA tissue were higher than that in normal tissue, prompting CA tissue cells in state of high proliferation.

### **CONCLUSION**

This experiment showed that NDRG1 and VEGF expression not only existed in CA tissue, and both of the expression were positively correlated, and indicated that both collaborative participated in the incidence and development of CA.

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