Correlation between nm23-H_1 expression and transvaginal color Doppler ultrasound performance after radiotherapy for cervical cancer

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Background: This study aimed to explore the correlation between nm23-H_1 gene expression and transvaginal color Doppler ultrasound performance following radiotherapy (RT) for cervical cancer.

Methods: Eighty patients with cervical cancer treated surgically after RT were retrospectively included in this study. Another 60 normal cervical tissue specimens from the pathology department and 30 cervical cancer tissues that did not undergo preoperative RT were included as control samples. Nm23-H_1 protein expression in cervical tissue was detected using immunohistochemistry. Color Doppler flow imaging (CDFI) was used to detect the imaging signs and resistance index (RI) of cervical cancer.

Results: (I) Immunohistochemical detection showed that the positive rates of the nm23-H_1 protein in cervical cancer and normal cervical tissues were 37.5% (30/80) and 70.0% (42/60), respectively, and the difference was statistically significant (χ^2=14.498, P=0.000). In cervical cancer cells, nm23-H_1 was mainly localized in the cytoplasm but was also expressed in small amounts in the cell membrane. (II) We observed that the expression status of nm23-H_1 was closely correlated with the tumor diameter, lymph node metastasis status, and differentiation degree of patients (P<0.05). The positive rate of nm23-H_1 was higher in patients with cervical cancer with larger tumor diameters, presence of lymph node metastasis, and lower differentiation degree. (III) Ultrasound findings in the 80 cervical cancer patients exhibited no echogenicity in two cases, moderate echogenicity in six cases, hypoechogenicity in 36 cases, and hyperechogenicity in 36 cases. The maximum RI of the patients' cervical tumors was 0.612, the minimum RI was 0.273, and the mean RI was 0.45. Nm23-H_1 protein expression was significantly correlated with CDFI grade and RI (P<0.05). Patients with positive nm23-H_1 expression had a significantly higher CDFI grade and RI compared to patients in the nm23-H_1 negative expression group.

Conclusions: Nm23-H_1 protein expression following RT for cervical cancer was significantly correlated with the clinicopathological parameters, CDFI grading, and RI index of cervical cancer patients.

Keywords: Cervical cancer; transvaginal color Doppler; radiotherapy (RT); nm23-H_1

Introduction

Cervical cancer is the second most common malignant tumor in women, with about 100,000 new cases in China annually, and an incidence rate that is increasing every year, with a significant trend toward younger patients (1). Currently, the main treatments for cervical cancer are surgery or concurrent radiotherapy (RT). Radical surgery is the treatment of choice in the absence of parametrial tissue invasion or distant metastases, and surgical treatment combined with postoperative adjuvant therapy can lead to radical cure for most patients (2). However, many patients with cervical cancer have local or regional spread of the tumor at the time of diagnosis, which deprives them of the opportunity for surgical treatment. In these cases, simultaneous RT becomes an important treatment option (3). One of the main mechanisms of the radiation effect is the induction of apoptosis and inhibition of tumor progression (4).

Nm23-H_1 is an important tumor suppressor gene located on chromosome 17p, which encodes the 53 kDa protein (5) and plays an important role in cell cycle regulation. Numerous researchers have highlighted the prognostic significance of nm23-H_1 in patients with various malignancies and determined that nm23-H_1 exerts oncogenic effects in malignant tumors (6). A study by Tee *et al.* (7) found that nm23-H_1 is involved in the formation of cervical cancer and is associated with some indicators of progressiveness. Overexpression of nm23-H_1 protein may indicate poor survival for cervical cancer patients. However, there are few studies regarding the correlation between nm23-H_1 gene expression and the effect of RT and ultrasound parameters in cervical cancer.

Therefore, in this study, we used immunohistochemistry to detect nm23-H_1 protein expression in tissues and transvaginal color Doppler ultrasound parameters following RT for cervical cancer. We also analyzed the correlation between nm23-H_1 protein expression and transvaginal color Doppler ultrasound performance following RT to provide new ideas for the clinical treatment and monitoring of cervical cancer. We present the following article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1114/rc).

Methods

Study subjects

Eighty cervical cancer patients admitted to The People's Hospital of Zhaoyuan City from January 2014 to January 2016 were retrospectively included in this study. The inclusion criteria were as follows: (I) patients diagnosed with cervical cancer by cytological or pathological examination; (II) it was confirmed by pathological diagnosis; (III) patients who had received preoperative RT; (IV) cases where the cancerous and paracancerous tissues were postoperatively preserved in our laboratory or pathology department; (V) patients with complete pre- and post-operative ultrasound and other imaging data. The mean age of the 80 included patients was (61.3±10.8) years. The main symptoms included contact bleeding, postmenopausal vaginal bleeding, and abnormal vaginal discharge. Another 60 normal cervical tissue specimens from the pathology department and 30 cervical cancer tissue specimens from cases that did not undergo preoperative RT were included as controls.

Preoperative assessment for cervical cancer involved routine blood and biochemical tests, liver and renal function tests, chest radiographs, cystoscopy, and abdominal CT examinations. All patients with cervical cancer were staged according to the International Federation of Obstetrics and Gynecology staging system. According to the pathological findings, there were 36 cases of squamous cervical cancer and 44 cases of adenocarcinoma. All patients received intracavitary brachytherapy and external RT in the RT unit, followed by biopsy or surgery. Preoperative RT was administered for 6 weeks, with the entire pelvis receiving 20 (five/week) external irradiation doses of 45 Gy. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The People's Hospital of Zhaoyuan City (No. 20210065). Individual consent for this retrospective analysis was waived.

Vaginal color Doppler ultrasound

We used the Acuson and ImagePoint HX ultrasound diagnostic instruments (Keyence, China) with transvaginal probes at 5.0–7.5 MHz. The patients were placed in a cystotomy position and a probe was inserted through the vagina into the fornix. Two-dimensional ultrasound was initially performed to visualize the ovaries, followed by a careful scan from the external to the internal cervical opening and the parametrial area to visualize the size of the cervix, the internal echo, the cervical mucosa, and part of the vagina. Blood flow was then visualized by color Doppler flow imaging (CDFI) and the resistance index (RI) was measured.

The CDFI grading was follows: Grade 0, no blood flow signal is detected within the mass; Grade I, a small amount of blood flow, with one to two punctate or thin rod-shaped tumor vessels visible; Grade II, a moderate amount of blood flow, with three to four punctate vessels or a longer vessel penetrating the lesion, which may approach or exceed the radius of the mass; and Grade III, a large amount of blood flow, with ≥five punctate vessels or two longer vessels visible. Grades 0 to I were considered to be benign lesions and grades II to III were considered to be malignant lesions. RI was calculated according to the formula RI = (Vs – Vd)/Vs, where Vs is the maximum flow rate during systole and Vd is the maximum flow rate during diastole of that vessel. An increase in RI indicated an increase in the RI of that vessel, suggesting the development of a corresponding lesion.

Hematoxylin-eosin (HE) and immunohistochemical staining

Tissue specimens were fixed in 4% formaldehyde, routinely dehydrated, and paraffin-embedded. Slices were cut into thin sections of approximately 4 μm and subjected to both HE and immunohistochemical staining. Sections were dewaxed in xylene and dehydrated in a gradient ethanol concentration, followed by microwave recovery of the antigen using a sodium citrate buffer solution, 3% H_2O_2 to block peroxidase, 10% serum blocking, and drop-wise co-incubation with the nm23-H_1 primary antibody (1:1,000 dilution, Abcam, USA) at 4 ℃ overnight. The next day, sections were washed three times with Phosphate Buffered Saline (PBS) and incubated with ready-to-use universal murine secondary antibody (Abcam) before being developed by diaminobenzidine (DAB) and photographed under a microscope (Leica, Wetzlar). We counted the number of positive cells, which were those with brown and dark brown nuclei.

Immunohistochemical scoring was conducted using a semi-quantitative method with four levels of staining intensity: 0 (no staining), 1 (weak staining), 2 (moderate staining), and 3 (strong staining). The positive cell area was expressed as a percentage (%): <25%, 0 points; 25–50%, 1 point; 51–75%, 2 points, and >75%, 3 points. The immunohistochemistry score was calculated according to the formula: total score = positive area value x intensity score, with a score ≥2 judged as positive expression. A ratio of the number of positive cells to the total number of cells in the visual field >5% was considered to indicate positive expression.

Statistical analysis

All data analysis was carried out using SPSS 20.0 software (IBM, America). Count data were expressed as a frequency (cases) and the chi-square test was used. P<0.05 was considered a statistically significant difference.

Results

Morphological characteristics of normal cervical and cervical cancer tissue by HE staining

No tissue anisotropy was observed in the normal cervical tissues. After RT, most cervical cancer tissues exhibited nuclear degeneration, nuclear lysis, cytoplasmic keratinisation (vacuolation) and regeneration, and fibrosis of the cancerous tissue. In non-radiated cervical cancer tissue, the tumor cells exhibited relatively normal morphology, and nuclear deformation and lysis were less common.

Immunohistochemical detection of nm23-H_1 protein expression in tissues

The immunohistochemical assay showed that the positive rates of nm23-H_1 protein in cervical cancer and normal cervical tissues were 37.5% (30/80) and 70.0% (42/60), respectively, and the difference was statistically significant (χ^2=14.498, P=0.000). In cervical cancer cells, nm23-H_1 was mainly localized in the cytoplasm but was also expressed in small amounts in the cell membrane. See *Figure 1*.

Effect of nm23-H_1 expression on the clinicopathological parameters of cervical cancer patients

Univariate analysis showed that nm23-H_1 expression status was closely correlated with the patients' tumor diameters, lymph node metastasis status, and differentiation degree (P<0.05), and the nm23-H_1 positive rate was higher in patients with cervical cancer with larger tumor diameters, presence of lymph node metastasis, and lower differentiation degree. However, there was no significant correlation between nm23-H_1 expression and the patients' age or pathological type (P>0.05). See *Table 1*.
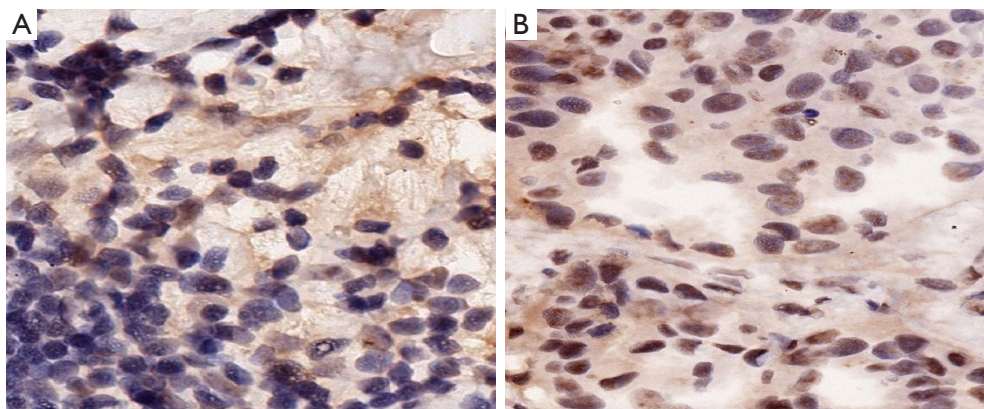
Figure 1 Expression of nm23-H_1 protein in cervical cancer tissues (×200, immunohistochemical staining). (A) Positive expression of nm23-H_1 in cervical cancer tissues. (B) Negative expression of nm23-H_1 in cervical cancer tissues.

Table 1 Effect of nm23-H_1 expression on clinicopathological parameters in patients with cervical cancer

Indicators	nm23-H_1 expression		χ^2	P
	Positive (n=30)	Negative (n=50)		
Age			0.014	0.907
≤40 years	17	29		
>40 years	13	21		
Tumor diameter			4.364	0.037
≤4 cm	18	18		
>4 cm	12	32		
Pathology type			0.054	0.816
Squamous carcinoma	13	23		
Adenocarcinoma	17	27		
Lymph node metastasis			4.566	0.033
Yes	10	29		
No	20	21		
Degree of differentiation			7.873	0.020
High	15	13		
Medium	10	14		
Low	5	23		

Transvaginal color Doppler ultrasound features in cervical cancer

Among the 80 cervical cancer patients, two cases exhibited no echogenicity, six cases showed moderate echogenicity (i.e., difficult to define morphology, tumor echogenicity close to the surrounding tissue, uniform internal echogenicity), 36 cases showed hypoechogenicity (i.e., cervical echogenicity lower than uterine body echogenicity, mostly homogeneous, clear lesion margins, solid masses in the neck with occasional strong echogenicity), and 36 cases exhibited hyperechogenicity (i.e., irregular morphology,

Figure 2 The uterus is anterior and atrophic. Uterus size: anterior-posterior diameter, 31 mm; upper and lower diameter, about 36 mm; and right and left diameter, 38 mm. After treatment for cervical cancer, the anterior-posterior diameter was about 30 mm, echogenicity was heterogeneous, the anterior wall thickness was about 16 mm, and the posterior wall thickness was about 15 mm. In combination with an MRI scan, lamellar hypoechogenicity was seen in the lower right posterior wall of the uterine cervix, the range was about 16×30 mm, the border was blurred, and no envelope is observed. CDFI showed a more abundant striped blood flow signal inside. MRI, magnetic resonance imaging; CDFI, color Doppler flow imaging.
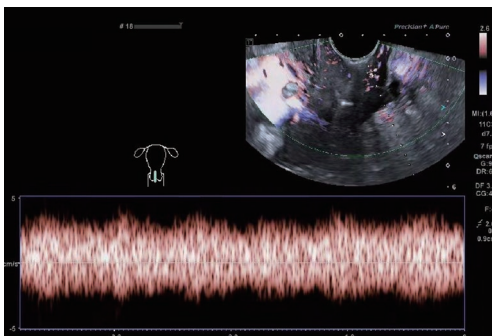

Figure 3 wo injections (both 1.5 mL) of Sonovue contrast medium via the peripheral intravenous group. Right posterior wall hypoechoic arterial phase of the lower cervical segment was about 12 s earlier than the anterior wall muscular layer from the periphery to start uneven slightly high enhancement, about 18 s to reach the peak, with a range of about 13×21 mm. After reaching the peak of the lesion, the boundary was not clear and the boundary of the surrounding tissue was divided, and it was clear that the lesion was hypoenhanced. In cases of hypoechoic echo and perfusion abnormalities in the right posterior wall of the lower cervical segment after RT for cervical cancer, consider alteration of the ultrasonographic image after RT for cervical cancer. RT, radiotherapy.

less light point enhancement, clear margins, slightly heterogeneous distribution).

Ultrasound examination revealed that in most patients the cervix was irregularly enlarged and the internal echogenicity was heterogeneous. Localized margins of the neck were irregular and poorly defined, and specific nodules occasionally appeared as strongly echogenic solid masses or sheet-like plaques. The cervical canal was poorly visualized, with a little tumor infiltration leading to obstruction of the cervical canal. There may also have been irregular anechoic areas of poor acoustic transmission in the pelvis. On CDFI, a rich blood flow signal was observed within the mass, usually showing a high velocity and low resistance arterial flow spectrum. Typical images are detailed in *Figures 2,3*.

Correlation of nm23-H_1 expression with CDFI classification and flow RI

The maximum RI of patients with cervical tumors was 0.612, the minimum RI was 0.273, and the mean RI was 0.45. Nm23-H_1 protein expression was significantly correlated with CDFI grade and RI ($P<0.05$). Patients with positive nm23-H_1 expression had a significantly higher CDFI grade and RI compared to those in the nm23-H_1 negative expression group (*Table 2*).

Discussion

Over the last 20 years, considerable progress has been made in the treatment of cervical cancer. In particular, the increasing use of intensity-modulated RT and image-guided brachytherapy has led to better patient prognosis and fewer treatment complications (8). Whether performed postoperatively in patients at risk of recurrence or as an initial curative treatment, RT is an integral part of the treatment of cervical cancer. Following radical hysterectomy, RT with simultaneous platinum-based chemotherapy is recommended for patients with high-risk factors, such as positive cut margins (9). For patients with intermediate-risk factors, postoperative RT is recommended. In cases of curative treatment, chemotherapy is recommended for patients with Federation International of Gynecology and Obstetrics (FIGO) stage IB3 to IVA disease. Curative RT or chemoradiotherapy is conditionally recommended for patients with medically inoperable stage IA1 to IB2 disease (10).

Table 2 Correlation of nm23-H_1 expression with CDFI classification and flow resistance index

Indicators	nm23-H_1 expression		χ^2	P
	Positive (n=30)	Negative (n=50)		
CDFI grading			12.411	0.000
0–I	7	32		
II–III	23	18		
RI			8.354	0.004
>0.4	22	20		
≤0.4	8	30		

CDFI, color Doppler flow imaging; RI, resistance index.

In this study, no tissue anisotropy was observed in normal cervical tissue using HE staining and immunohistochemical detection. After RT, most cervical cancer tissues showed nuclear degeneration, nuclear lysis, cytoplasmic keratinisation and regeneration, and fibrosis of the cancerous tissue. In cervical cancer tissues without RT, the tumor cells exhibited relatively normal morphology, and nuclear deformation and cell lysis were less common. Meanwhile, the positive rates of nm23-H_1 protein in cervical cancer and normal cervical tissues were 37.5% and 70.0%, respectively. An imbalance of signaling pathways in cervical cancer cells may lead to cell transformation and cancer development.

The wild-type nm23-H_1 protein is a transcription factor, which when activated in the G1/G2 phase, temporarily inhibits cell division and repairs damaged Deoxyribo Nucleic Acid (DNA), and induces apoptosis when DNA damage cannot be repaired (11). Zhao *et al.* (12) found that nm23_H1 negatively regulated TGF-β1-dependent induction of epithelial-mesenchymal transition (EMT) in non-invasive lung cancer cell lines. When nm23_H1 was downregulated, epithelial cell marker E-cadherin, mesenchymal cell marker β-catenin, and fibronectin were upregulated, and this series of changes was associated with enhanced expression of TGF-β1 after nm23_H1 downregulation. Overexpression of the nm23-H_1 protein is significantly correlated with the survival of cervical cancer patients after RT, suggesting that nm23-H_1 plays an important role cervical cancer RT. Considering that most cervical cancers are associated with human papillomavirus (HPV) infection, nm23-H_1 can be inactivated during cervical carcinogenesis via formation of complexes with the HPVE6 oncoprotein, thereby accelerating tumor progression. A recent study

has shown that cervical cancer cells exposed to RT have significantly increased levels of nm23-H_1 protein, leading to G1 phase cell cycle arrest and apoptosis (13). Researchers have concluded that the wild-type nm23-H_1 protein is overexpressed before RT, mutant nm23-H_1 is overexpressed after RT, and the conversion process of the nm23-H_1 gene from wild-type to mutant is attributable to RT, where each nm23-H_1 exon contains frequently mutated sites and RT can directly induce the production of nm23-H_1 mutation.

In this study, using the chi-square test, we found that patients with positive nm23-H_1 expression had a significantly higher CDFI grade and RI compared to patients in the nm23-H_1 negative expression group. Transvaginal color Doppler ultrasound provides an objective and comprehensive interior view of the cervical cancer, uterine cavity, bladder, and rectum, as well as their blood flows (14). In this study, there were no significant changes in the morphology of the cervix in the early stages of cervical cancer and no positive transvaginal color Doppler ultrasound findings. As the cervical cancer progressed, the cervix showed irregular enlargement, manifesting as a rough and uneven surface. Transvaginal color Doppler ultrasound revealed localized irregularities in the margins of the cervix with less well-defined borders, occasionally with specific nodules appearing as strongly echogenic solid masses or lamellar plaques (15). The cervix was shoe-like and hypertrophied, with heterogeneous internal echogenicity. The cervical canal was poorly visualized, with a little tumor infiltration leading to cervical canal obstruction and possible regular anechoic areas of poor acoustic transmission in the pelvis.

CDFI facilitates preoperative assessment of the blood flow signal within the cervical cancer from a macroscopic

perspective, which can be aided by MRI (16). In the present study, CDFI demonstrated that there was little blood flow signal in normal cervical tissue and abundant blood flow signal in cervical cancer tissue, often showing a high velocity, low resistance arterial blood flow spectrum and a high velocity, high resistance blood flow spectrum in the presence of co-infection. Two-dimensional ultrasound shows different signs of exophytic cervical cancer, endophytic cervical cancer, and cervical cancer invading the uterus and bladder (17). With the development of ultrasound technology and the accumulation of experience in clinical application, transvaginal color Doppler ultrasound has become an important tool for the adjuvant diagnosis of mid- to late-stage cervical cancer. However, this study also has shortcomings. This study is a retrospective analysis, which is likely to cause some deviations in the results. Our next step will be to conduct multi-center clinical trials to validate these results.

In summary, this study found that nm23-H_1 protein expression following RT for cervical cancer was significantly correlated with cervical-occupying lesions and tumor size, as revealed by transvaginal color Doppler ultrasound, CDFI grading, and RI. Therefore, nm23-H_1 levels and transvaginal color Doppler ultrasound may provide some valuable clinical data for the treatment and monitoring of cervical cancer.

Limitations

We failed to study the biological function of nm23-H_1 in cervical cancer. This study is a retrospective analysis, which is likely to cause some deviations in the results. It needs to be further confirmed by multi-center clinical trials.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1114/rc

Data Sharing Statement: Available at available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1114/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-22-1114/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Ethics Committee of The People's Hospital of Zhaoyuan City (No. 20210065). Individual consent for this retrospective analysis was waived.

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