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Pathomorphological Markers of Overcoming Radioresistance in the Treatment of Cervical Cancer

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Abstract

The aim of this study was to study pathomorphological markers of decreasing radioresistance of cervical tumor tissue during radiation therapy and the administration of sodium deoxyribonucleate.

Methods and Results: The object of the study was patients with diagnosed cervical cancer (T1/FIGOIB, T1a1-2/FIGOIA1-2, and T1b1-2/FIGOIB1-2; NX0 and M0). All patients in the study were divided into 2 groups. The main group (MG) included 40 patients receiving combination of standard chemoradiotherapy with sodium deoxyribonucleate (5ml intramuscularly for 20 days). The comparison group (CG) included 23 patients receiving only standard chemoradiotherapy. Histological and immunohistochemical analysis was performed.

Conclusion: Against the background of complex therapy, the TLR9 expression indices in the patients of the MG were 24%-36% higher than the indices in the patients of the CG. The inclusion of sodium deoxyribonucleate—a TLR9 agonist—in the chemoradiotherapy regimen for cervical cancer has great potential for stimulating the TLR9 expression in immunocompetent cells of the tumor microenvironment. **(International Journal of Biomedicine. 2020;10(2):120-123.)**

Key Words: radioresistance • TLR9 expression • cervical cancer • pathomorphological markers

Introduction

The possibility of increasing the radiosensitivity of tumors is of high practical importance in oncology.[1-3] In radiation therapy, of particular relevance is the search for chemical compounds that not only increase the sensitivity of tumor tissue to the effects of radiation therapy, but also act indirectly, by activating their own immunocompetent cells, including in the area of the tumor microenvironment.[4,5]

One of the ways to implement the above strategies is to analyze the expression of a group of cell receptors of the innate immune response, known as the family of PRRs (pathogen recognition receptors), capable of recognizing and binding to antigens of pathogen-associated molecular patterns.[2,5] The main PPR family is the Toll-like receptors (TLRs), which recognize molecular patterns associated with pathogens, including bacteria, viruses, fungi and protozoa.[6-8]

TLRs are largely classified into 2 subfamilies based on their localization: cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) and intracellular TLRs localized in the endosome (TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13).[9,10] TLR9 is activated intracellularly by bacterial DNA and synthetic oligodeoxynucleotides containing unmethylated CpG dinucleotides (CpG motifs).[6,11]

As a rule, TLR activation leads to the production of cytokines and antimicrobial factors through common intracellular signaling pathways. The TLR family harbors extracellular leucine-rich repeat domains and a cytoplasmic domain that is homologous to that of the interleukin (IL)-1 receptor (IL-1R) family. After stimulation, TLR recruits IL-1R-associated kinase via adaptor myeloid differentiation factor 88 (MyD88) and induces activation of NF-kappaB and mitogen-activated protein kinases.[3,4,6,12] The TLR9-MyD88 signaling pathway plays a critical role in promoting adaptive immune responses and that modulation of this pathway may have enormous therapeutic potential in enhancing vaccine potency, controlling autoimmunity, as well as improving the outcome of viral vector-mediated gene therapy.[13]

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The search for and study of TLR9 agonists is of great scientific and practical importance. According to the literature, sodium deoxyribonucleate can be classified as an agonist to TLR9.[14,15] Thus, the mechanisms of the immunotropic effect of sodium deoxyribonucleate, which contains unmethylated CpG motifs, suggest a high tropism for TLR9 with subsequent activation of innate immunity mechanisms. The rationale for investigating TLR9 agonists as antitumor agents is based on the hypothesis that the innate immune response may have direct antitumor effects. A study of the expression of TLR9 against the background of the use of sodium deoxyribonucleate may be useful to clarify the mechanism of the sodium deoxyribonucleate action at the level of the tumor microenvironment.

The aim of this study was to study pathomorphological markers of decreasing radioresistance of cervical tumor tissue during radiation therapy and the administration of sodium deoxyribonucleate.

Materials and Methods

The object of the study was patients with diagnosed cervical cancer (T1/FIGOIB, T1a1-2/FIGOIA1-2, and T1b1-2/FIGOIB1-2; NX0 and M0). All patients in the study were divided into 2 groups. The main group (MG) included 40 patients receiving combination of standard chemoradiotherapy with sodium deoxyribonucleate. The comparison group (CG) included 23 patients receiving only standard chemoradiotherapy. The standard treatment protocol included radiation therapy (RT) in combination with weekly platinum chemotherapy (CT). RT: median doses to point A and B were 85–90 Gy and 55–60 Gy, respectively. Cisplatin 40 mg/m^2 with gemcitabine 125 mg/m^2 was prescribed weekly against the background of the remote component of RT, then after RT, 2 courses of adjuvant CT (cisplatin 50 mg/m^2 on Day 1 and gemcitabine 1000 mg/m^2 on Days 1 and 8 of a 21-day cycle with an interval of 3 weeks) were performed.

During the study period, all patients were given a threefold morphological study of biopsy material: biopsy #1 (biopsy before treatment), biopsy #2 (14 days after treatment) and biopsy #3 (28 days after treatment). A 1.5% solution of sodium deoxyribonucleate (5 ml daily) was administered intramuscularly for 20 days. For routine staining of histological preparations, Mayer hematoxylin and a 1% alcohol solution were used. Immunohistochemical analysis of TLR9 expression was performed using monoclonal antibodies (Anti-TLR9 antibody [26C593.2] ab134368 (100 μg) (manufacturer: CarlZeiss Microscopy, Germany). Tissue samples were processed using the AGT11-FMP-4 system and BenchMark XT immunostainer.

Unmasking was carried out on the Dako PT Link using EnVisionTM FLEX Target Retrieval Solution, High pH (50x Tris/EDTA buffer, pH 9) (Dako Omnis); heating time of 20 minutes (t=95°C). We used the Histofine imaging system (the polymer incubation time of 20 minutes, t=22-24°C), Dako chromogen (incubation time of 10 minutes with 22-24°C, without amplification). Sections were prepared using a Slide 4004M sled microtome. Morphometry of the preparations was carried out with an Olympus microscope BX2WI; for photo documentation, we used a hardware-software complex for biological research with a documenting system based on the upright microscope Axio Imager-2 (Carl Zeiss, Germany).

Quantification of the expression level of TLR9-positive cells in the cervical biomaterial was performed by determining the representation of TLR9-positive immunocompetent cells in the field of view using an x40 lens. During a planimetric analysis, the entire volume of biopsy material was examined; the number of fields of view could be more than 40. The results were expressed quantitatively in the average amount of expression of TLR9-positive cells in the field of view of each patient. Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA).

Results and Discussion

Comparison of biopsy material in patients of the CG and MG groups before treatment showed that the tumor was represented by squamous cell carcinoma (a well-differentiated tumor in 65% and 58% of cases, respectively; moderately differentiated in 35% and 42% of cases, respectively). Tumor cells were represented by layers of atypical squamous epithelium with the phenomena of invasive and infiltrative growth and mitoses, including pathological ones. The study of biopsy material during therapy showed grade IV therapeutic pathomorphosis in all cases in the MG and 84% of cases in the CG. Tumor regression was combined with pronounced fibrotic changes: 24% in the CG and 46% in the MG. In the MG, in all patients who underwent biopsy #3, there was an almost total regression (97%) with a pronounced picture of fibrosis. It is worth noting that dystrophic groups of tumor cells in the CG were rarely encountered during the third biopsy. Thus, in both groups, against the background of the therapy, a significant regression in the volume of the tumor lesion and an increase in the amount of connective tissue (fibrosis) were revealed. Slight differences were observed in the severity of changes on biopsies #1 and #2: in patients of the MG, tumor regression was observed 16% more often than in patients of the CG.

Before treatment, cells with different degrees of immunopositivity for TLR9 were detected in patients of CG and MG in the biopsy material (Photos 1A, C). The relative content of cells with high TLR9 expression was small (Photo 1A) and tissue distribution (Photo 1B) was uneven. Radiation therapy led to increasing the TLR9 expression in tumor stromal cell cytoplasm (Table 1) in both study groups. At the same time, in patients of the MG by the time of biopsy #3, a higher level of TLR9 expression in the biopsy material was noted (Table 1). In the MG after treatment, a visual increase in cells with high immunopositivity for TLR9 was noted, not only peritumorally (Photos 2C,D) but also intratumorally (Photos 2A,B).

Table 1.

Comparative indicators of TLR9 expression in patients of compared groups (CU)

Observation period	CG	P-value	MG
Biopsy #1	2.95±0.15	<0.05	3.15±0.05
Biopsy #2	4.15±0.15	<0.05	4.65±0.15
Biopsy #3	3.95±0.05	<0.01	5.15±0.05

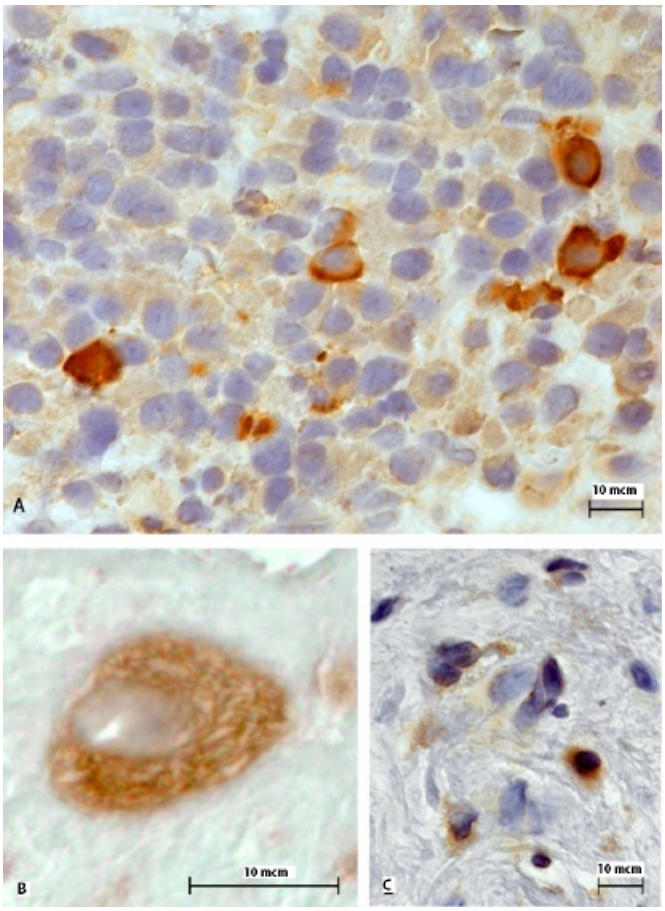

Photo 1. *Expression of TLR9 by tumor microenvironment cells in the cervical biomaterial before treatment: A - tumor microenvironment cells with a high TLR9 content; B - cytological picture of tumor stromal cells, which are immunopositive for TLR9 cells; B - different intensities of TLR9 expression in tumor stromal cells.*

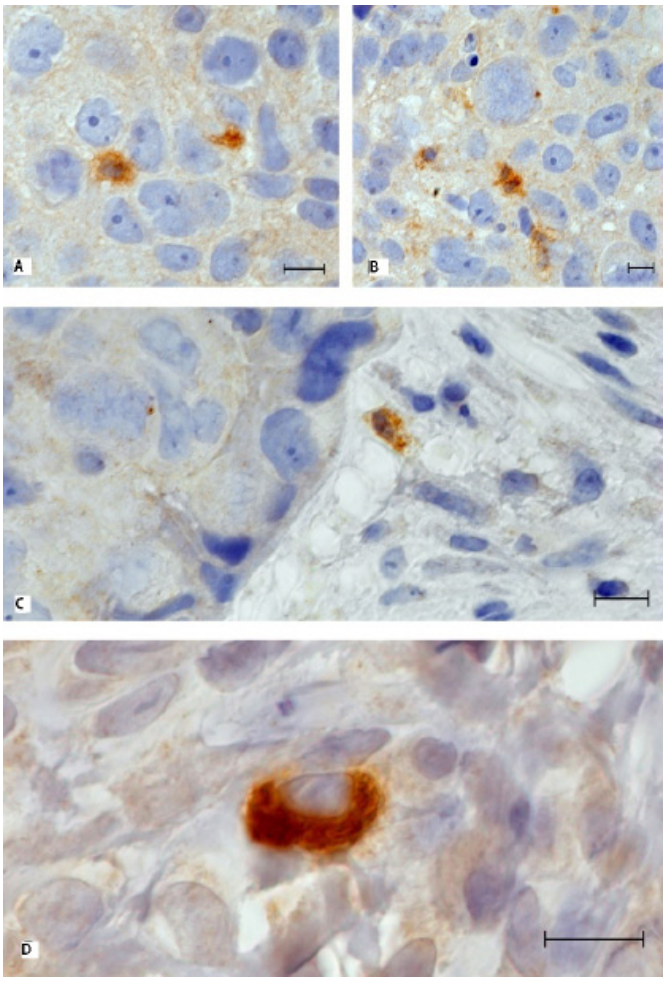

Photo 2. *Expression of TLR9 by tumor microenvironment cells in the MG: A, B — localization of TLR9 positive cells in close proximity to tumor cells; C – a localization of TLR9 positive cells in the peritumoral region; D – a cell with a high degree of immunopositivity for TLR9 in the tumor stroma.*

Thus, against the background of complex therapy, the TLR9 expression indices in the patients of the MG were 24%-36% higher than the indices in the patients of the CG. The inclusion of sodium deoxyribonucleate—a TLR9 agonist—in the chemoradiotherapy regimen for cervical cancer has great potential for stimulating the TLR9 expression in immunocompetent cells of the tumor microenvironment.

Competing Interests

The authors declare that they have no competing interests.

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